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Gene and Chromosome Homology in Fishes of the Genus *Xiphophorus*[1]

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(Plates 1-6; Text-figure 1)

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I. INTRODUCTION

THE SIMILARITY of closely related species is primarily the result of their descent from a common ancestor, and there can be little doubt that most of the gene loci in such species are the same, although they are often occupied by different alleles. *Identical alleles* may be defined as alleles that have been inherited from the ancestral form by two or more descendent species or populations. *Homologous alleles* are genes that occupy the same locus in different species; often they are alleles that have arisen by mutation in one species but not in another. In contrast, *analogous genes* are those that have, or seem to have, the same function or effect, but that cannot be traced to a common locus. A fine distinction between identical and homologous alleles is not always possible. An allele may be the same in two species because the same mutation occurred in both of them, rather than because it was inherited from a common progenitor—in which case the two genes would be homologous, even though identical in structure. Moreover, alleles that have different nucleotide sequences can give rise to the same phenotypic effect. Such alleles should be considered homologous, but unless a molecular analysis is made, which is at present possible in very few cases, they will be considered identical.

Under the usual circumstances, genes can be studied only indirectly by their phenotypic effects, and most characters are not governed by

a single major or principal gene, but by the interaction of many, individually unidentifiable genetic factors. Even within a single species, the same structure or function may be the result of different combinations of genes in different individuals, because of gene substitutions and repressor mutations—a phenomenon called the "constancy of the phenotype" by Mayr (1963, p. 280). In different populations of the same species, as well as in closely related species, identical characters may be based upon different polygenic mechanisms; in these cases, even though the characters themselves may be homologous, their genetic basis cannot be considered so (de Beer, 1958, p. 148). Therefore, in our present state of knowledge, the only characters suitable for the study of gene homology are those under the control of a single major gene that exists in at least two recognizably different allelic states. Strikingly similar mutations that have arisen in closely related species and similar multiple allelic series that occur in congeneric species have provided some of the most favorable material for the study of gene homologies.

This paper is concerned with gene and chromosome homologies in the well-known genus of teleost fishes, *Xiphophorus*. Within this genus several taxonomic levels are represented: (1) geographically isolated populations belonging to a single species, (2) morphologically recognizable subspecies, (3) species, (4) superspecies, and (5) less closely related species groups (Rosen, 1960). Because the members of all of these are interfertile to an appreciable degree, critical genetic experiments that require hybridization can be performed. Roughly corresponding to their taxonomic relationships, these fishes exhibit strikingly similar or different patterns of pigmentation, some of which are polymorphic and are controlled by major genes. Most notable are the tail-spot patterns, composed of small melanophores (micromelanophores) in aggregations near the base of the caudal fin, and the macromelanophore patterns, formed by large pigment cells (often 0.3 to 0.5 millimeters in diameter) that may occur on almost any part of the body. The macromelanophore genes are physiologically similar in that, with a single exception, they are capable of giving rise to pigment cell abnormalities in hybrids. Four species of *Xiphophorus* have populations that are polymorphic for both macromelanophore and tail-spot patterns, one species for only tail-spot patterns, another for only macromelanophore ones, and two species exhibit neither type of pattern. In *Xiphophorus maculatus*, which is the most polymorphic species, at least 16 different alleles have been recognized, half at the macromelanophore and half at the tail-spot locus. Moreover, the less well-known species, *X. variatus*, may prove to be just as phenotypically diverse. The other four polymorphic species exhibit relatively few pigment patterns, but the genus as a whole provides a remarkable gamut of opportunities to study gene homology.

II. Materials and Methods

In the first part of this paper, the macromelanophore patterns of *Xiphophorus* and the genes responsible for them are briefly reviewed and the morphology and inheritance of several new patterns are described. In the second part, the micromelanophore patterns are treated in the same way. Crosses with a critical bearing on the question of gene homology are analyzed in the third.

At the present time, several laboratories are studying the pigment patterns of *Xiphophorus*, and a uniform system of nomenclature to describe the different patterns and alleles is needed. One difficulty is that many of the patterns are not well known and that sufficient comparative material is often not available. Another source of error has been that many of the stocks of *Xiphophorus* have been obtained from commercial sources. The geographical origins of these fish are unknown. Moreover, many of the domesticated stocks represent not pure species, but fish descended from interspecific hybrids. For example, there is little doubt that the striking red and black pigment patterns of swordtails regularly available in the pet trade are the result of genes belonging to other species of *Xiphophorus* that have been introduced into *X. hellerii* through introgressive hybridization. Other commercial stocks have hybrids between *X. maculatus* and *X. variatus* as their basis. Some of these resemble *variatus*, but have *maculatus* pigment genes and vice versa.

The geographic origin of the fish in this report and the expedition responsible for their collection are listed below:

Xiphophorus couchianus couchianus (Girard, 1859).

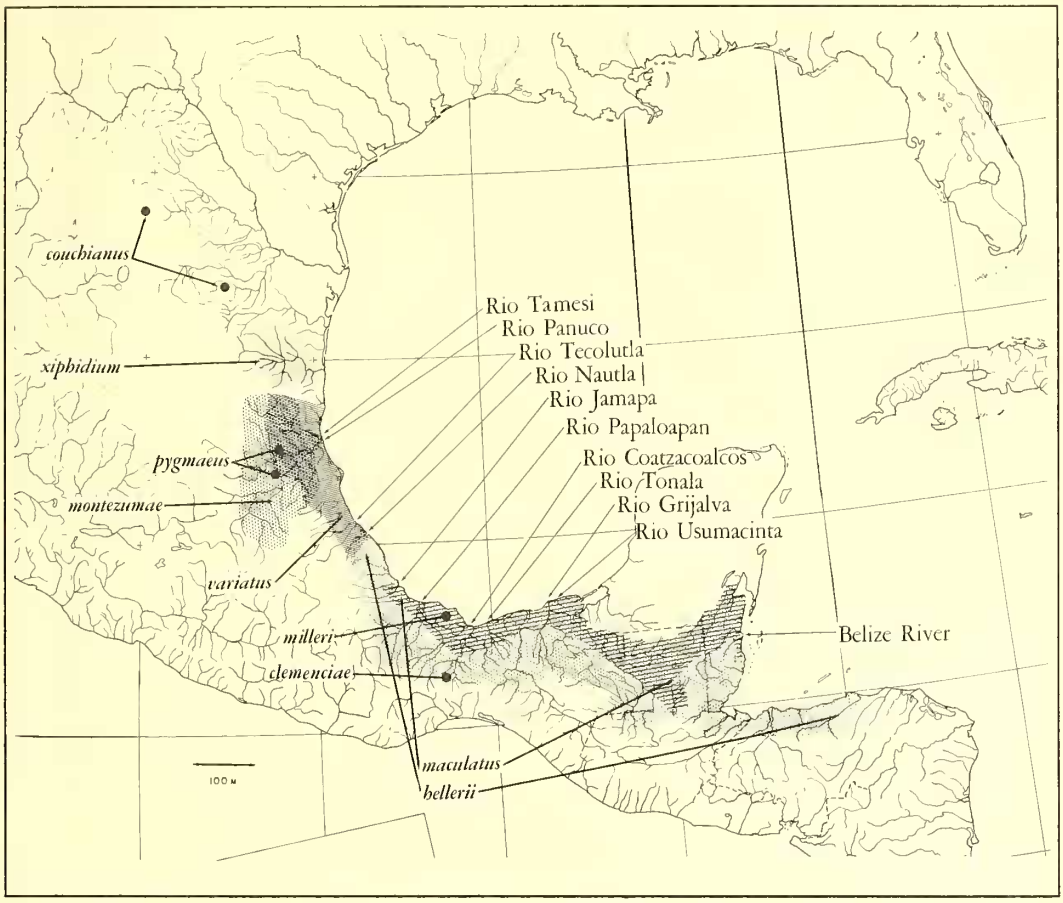
Pedigree h-28: Rio Santa Catarina, Nuevo Leon (1939) Myron Gordon, Atz, Evelyn Gordon. Hybrids with *X. v. xiphidium*.

Strain Xc-G: Rio Santa Catarina, Nuevo Leon (1958) Myron Gordon, Evelyn Gordon.

Xiphophorus variatus xiphidium (Gordon, 1932).

Pedigrees Px-20 to 23: Rio Purification, Tamaulipas (1939) Myron Gordon, Atz, Evelyn Gordon.

Pedigrees h-2, h-28: Rio Purification, Tamau-



TEXT-FIG. 1. The distribution of *Xiphophorus* (based principally on Rosen, 1960). *X. v. variatus, X. montezumae* (with two subspecies), *X. maculatus,* and *X. hellerii* (three of the four subspecies) have the widest distribution. Not indicated on the map are the ranges of *X. v. evelynae,* which is restricted to headwater streams of the Rio Tecolutla, and *X. hellerii alvarezi,* which is known only from the Rio Santa Domingo, a tributary of the Rio Usumacinta in the state of Chiapas, Mexico.

lipas (1939) Myron Gordon, Atz, Evelyn Gordon. Hybrids with *X. v. variatus* and *X. c. couchianus.*

Pedigree 1184: Rio Santa Engracia, Tamaulipas (1958) Myron Gordon, Evelyn Gordon.

Pedigree 1228, 1238: Rio Santa Engracia, Tamaulipas (1962) Kallman.

Xiphophorus variatus variatus (Meek, 1904)

Pedigrees h-2, h-61: Rio Axtla, San Luis Potosi (1939) Myron Gordon, Atz, Evelyn Gordon. Hybrids with *X. v. xiphidium* and *X. maculatus.*

Pedigree 1752: Rio Tamesi, Tamaulipas (1957) Rosen, Malcolm Gordon, Myron Gordon.

Pedigree 912: Rio Tamesi, Tamaulipas (1957) Rosen, Malcolm Gordon, Myron Gordon. Hybrids with *X. v. evelynae.*

Xiphophorus variatus evelynae Rosen, 1960.

Pedigree 912: Rio Necaxa, Puebla (1957) Rosen, Malcolm Gordon, Myron Gordon. Hybrids with *X. v. variatus.*

Xiphophorus montezumae montezumae Jordan & Snyder, 1900.

Pedigree 733: Rio Salto, San Luis Potosi (1957) Rosen, Malcolm Gordon, Myron Gordon.

Pedigree 1817: Rio Salto, San Luis Potosi (1965) Klaus Kallman, Judith Kallman.

Xiphorphorus montezumae cortezi Rosen, 1960.

Pedigrees Xmc-21 to 29 and descendants,

Strain 38: Rio Axtla, San Luis Potosi (1939) Myron Gordon, Atz, Evelyn Gordon.

Xiphophorus milleri Rosen, 1960.

Pedigree 1374: Lake Catemaco, Veracruz (1963) Kallman, Rosen.

Pedigree 1543: Lake Catemaco, Veracruz. Obtained in 1963 through the courtesy of Dr. Robert R. Miller, University of Michigan.

Xiphophorus maculatus (Guenther, 1866).

Strains Jp 30, Jp 163 A and B: Rio Jamapa, Veracruz. Pure-line, inbred.[2]

Strain Gp: Rio Grijalva, Tabasco. Pure-line, inbred.[2]

C-30: a sub-line of Jp 30.

Strains Hp-1, Hp-2: Rio Hondo, British Honduras. Pure-line, inbred.[2]

Fish Cp-11: Rio Coatzacoalcos (1948) Myron Gordon, Atz, F. G. Wood, Jr. A single male.

Strain Np: New River, British Honduras (1954) Myron Gordon, Fairweather, Chaveria.[2]

Pedigree 1342: Rio San Pedro de Martir (1963) Kallman, Rosen.[2]

Pedigree 1900: Belize River, British Honduras (1966) Klaus Kallman, Judith Kallman.

Xiphophorus hellerii hellerii.

Strain Cd: Cordoba, Rio Jamapa, Veracruz. Obtained in 1949 through the courtesy of Dr. Reeve M. Bailey, University of Michigan. Originally collected by Dr. Clarence L. Turner.

Xiphophorus hellerii strigatus Regan, 1907.

Strain 3B: Arroyo Zacatispan, Rio Papaloapan, Oaxaca (1939) Myron Gordon, Atz, Evelyn Gordon.

Strain Cx: Near Almagres, Rio Coatzacoalcos, Oaxaca (1948) Myron Gordon, Atz, F. G. Wood, Jr.

Pedigree 1377: Rio Sarabia, Oaxaca (1963) Kallman, Rosen.

Xiphophorus hellerii guentheri Jordan & Evermann, 1896.

Strain Bx: Belize River, British Honduras (1949) Myron Gordon, Fairweather.

Strain Hx: Rio Lancetilla, Honduras (1951) Myron Gordon.

Strain Gx: Rio Grijalva, Tabasco (1952) Myron Gordon.

These fishes were bred and maintained at the Genetics Laboratory according to the method of Gordon (1950a) and Kallman (1965a). Most of them were eventually preserved either in formalin or alcohol to make them available for future reference.

III. Results

1. The Macromelanophore Patterns.

a. *Xiphophorus maculatus.*

The macromelanophore patterns of this species have been studied in greater detail than those of any other member of the genus. Five macromelanophore patterns have been described by Gordon (1948, 1951c) and Gordon & Gordon (1957) from natural populations: spotted *(Sp)* with irregular spotting along the flanks; striped *(Sr)* with discrete rows of macromelanophores, some of which are combined to form spots, along the flanks; spotted dorsal *(Sd)* with irregular spotting in the dorsal fin; nigra *(N)* with irregular blotches or bands on the flanks; and spotted belly *(Sb)* with heavy spotting on the ventral half of the body, especially in the area above the base of the anal fin. All fish with spotted belly are descended from a single male collected in 1932 from the Rio Papaloapan, the only one of its kind ever seen in nature (Gordon, 1946a). Two patterns that are known only from domesticated stocks of unknown geographic origin have been studied in some detail: fuliginosus *(Fu)* in which the fish are covered more or less uniformly by macromelanophores and have a sooty appearance (Kosswig, 1938; Gordon & Baker, 1955; MacIntyre, 1961a; Öktay, 1954), and a type of spotted pattern *(Sp')* that produces a pepper-and-salt effect (Gordon, 1951b). The phenotypic expression of the macromelanophore genes is greatly influenced by genetic modifiers (Gordon, 1951a; Gordon & Gordon, 1957). The phenotypic variation shown by the nigra pattern, however, may not result solely from modifying genes, since Bellamy & Queal (1951) recognized two additional alleles, thin nigra (N^t) and extended nigra (N^e). Unfortunately, they never described their complete experiments nor provided photographs of the patterns.

There is abundant evidence that the macromelanophore patterns in *X. maculatus* are controlled by dominant, sex-linked alleles (Bellamy, 1922; Bellamy & Queal, 1951; Gordon, 1927, 1937a, 1947a, 1951c, 1952; Kallman, 1965a; Öktay, 1959a, b, 1962). Two cases of crossing over within the macromelanophore locus have been recorded (Gordon, 1937a; MacIntyre, 1961c), and this suggests that the macromelanophore genes form a super-gene or a pseudoallelic or suballelic series.[3]

[2] The origin of these strains has been explained in detail by Kallman (1965a).

[3] The proper term for this situation presents a problem. Atz (1962) called these macromelanophore genes *pseudoalleles,* but this term has been reserved for an

In *X. maculatus*, the macromelanophore gene is closely linked to a locus controlling the appearance of yellow, orange, and red pigment patterns. Crossing over between this and the macromelanophore locus occurs in rare cases (Fraser & Gordon, 1929; Gordon, 1937a, 1950b). Breider (1936, 1938) and Kosswig (1948) offered the opinion that *Sp, N, Sb, Fu, Dr, R, Mo, Rb*, and *RSp* are all alleles, the last five of which concern patterns with red or reddish pigmentation. For most of these, however, no critical crosses demonstrating homology are available.

b. Xiphophorus variatus.

The pigmentary polymorphism of *X. variatus* appears to be as great as that of *X. maculatus*, but it has not yet been studied in detail. In the subspecies, *X. v. xiphidium*, there is a macromelanophore pattern, flecked *(Fl^1)*, that produces sharply defined, large, jet-black spots along the flanks of the fish (Gordon & Smith, 1938, fig. 7B).[4] This spotted pattern is apparently identical with the one studied by Zander (1962) and Anders & Klinke (1965), as judged by their descriptions and photograph. A second macromelanophore pattern is represented abundantly in populations inhabiting the Rio Santa Engracia (see Fig. 12). Its overall appearance is somewhat intermediate between *Sr* and *Sp'* of *X. maculatus*. In adult fish, the macromelanophores typically are not arranged in spots, but instead follow rather closely the reticulum (Rosen, 1960, p. 180; Atz, 1962, p. 156) that is formed by bands of micromelanophores along the edges of the scale pockets. Especially in the area below the dorsal fin and on the caudal peduncle, the macromelanophores may completely replace the reticular micromelanophores. In many cases macromelanophores have also "spilled over" into the hexagonal or rhombic areas that are usually free of melanophores. Nevertheless, three distinct rows of macromelanophores can almost always be distinguished: along the midlateral line and the two horizontal scale rows immediately above it. Anterior to the dorsal fin, this pattern is represented by numerous isolated macromelanophores or small elongated spots on the reticulum. Fish in which this pattern is strongly developed appear dusky, but never black, and the name assigned to it is based on this appearance, namely dusky, but we designate it as Fl^2 in accordance with our system of not trying to give a separate and appropriate name to every different but related pattern.[5] In heterozygous fish, Fl^2 masks the heavy spotting of Fl^1. When an Fl^1 Fl^2 female (phenotypically dusky) was mated to wild type male, the two pigment patterns segregated among the offspring of both sexes (Table I, ped. 1320). When a spotted female was mated to a dusky male, the dusky pattern *(Fl^2)* was inherited only by the female offspring while the males were of two types, Fl^1 and wild type (Table I, ped. 1324). The two spotted patterns must be caused by different sex-linked alleles and are not the result of the action of modifiers on a single macromelanophore gene. In our stocks, both genes are located on *X* chromosomes; in the stock of Kosswig (1959), Zander (1962), and Anders & Klinke (1965), the Fl^1 gene is on the *Y* chromosome.

We cannot trace *Pu*, a third macromelanophore pattern of *xiphidium* that was mentioned by Kosswig (1948, p. 142), but if this investigator is referring to the work of Myron Gordon that was first reported in Gordon & Smith (1938), it must be the gene we call Fl^1.

In another subspecies, *X. v. variatus*, several macromelanophore patterns occur in nature (Rosen, 1960, pp. 80-81), but only a few have been studied in the laboratory. The pattern punctatus, *P*, which was described by Kosswig (1935 a, b) and which we designate as P^1, consists typically of numerous black spots that are primarily located above the midlateral line and are most numerous below and in front of the dorsal fin, as judged by the photographs in Kosswig (1935 a, b) and Rust (1939) and an outline drawing by Zander (1962) and by direct comparison with wild-caught fish described in Atz (1962, p. 162). A similar pattern is present in one of the stocks of the Genetics Laboratory and is probably caused by the same allele, P^1. The original fish with this pattern were collected in the Rio Boquilla of the Rio Tamesi drainage.

obviously different relationship among genes, and the macromelanophore genes might better be designated *suballeles* according to the criteria of Serra (1965). Since nothing is known about the fine structure of the chromosomes of *Xiphophorus*, however, the exact type of multiple allelism that is present must remain a question. On the other hand, whether or not the macromelanophore alleles represent a super-gene, as defined by Ford (1964, pg. 93), is also at present unknown. Nevertheless, because it seems most likely that these genes "act as a switch in the control of polymorphism," we shall consider them as parts of a super-gene that, on rare occasions, may be separated by crossing over.

[4] Called *Sp* by Gordon & Smith (1938), Gordon (1943), Kosswig (1959), Zander (1962), and Anders & Klinke (1965); also by Atz (1962), but see the following footnote.

[5] In Atz (1962), crosses 903, 913, and 914, and fig. 10 concern dusky (Fl^2); the remaining crosses that involve a spotted *X.v. xiphidium*, including 941, concern Fl^1, as far as known.

TABLE 1. INHERITANCE OF TWO MACROMELANOPHORE PATTERNS, *Fl*[1] *and Fl*[2], IN *Xiphophorus variatus xiphidium*

Pedigree	Parents		Offspring					
	Female	Male	Females			Males		
			Fl[2]	*Fl*[1]	+	*Fl*[2]	*Fl*[1]	+
1251	1184-½ Fl[2]	1228-12 Fl[1]	30			22		
1281	1238-1 +	1228-11 Fl[1]		26				22
1320	1251-½ Fl[2] (Fl[1])	1281-11 +	20	19		6	10	
1324	1281-½ Fl[1]	1251-11 Fl[2]	25				17	15
1379	1281-¾ Fl[1]	1281-12 +		5	9		10	3
1499	1379-½ Fl[1]	1379-11 Fl[1]		21			13	5
1647	1499-½ Fl[1]	1499-11 +		10	3		11	3
1658	1499-4 Fl[1]	1499-12 Fl[1]		11			15	
1711	1647-1 Fl[1]	1647-11, +		5	3		5	5
1810	1711-½ +	1711-11 Fl[1]		12				17

The punctatus pattern is strikingly different from another spotted pattern, P^2, that causes large, intensely black spots most numerous along the midlateral line. These spots may coalesce to form an irregular black band in older fish see (Fig. 1).[6] These two punctatus patterns are usually impossible to tell apart in younger individuals, and not until a brood of fish reaches an age of, say, nine months can all its members be classified. There is, however, very little or no phenotypic overlap between the two patterns, once they have fully developed. This holds true despite the fact that old P^1 fish may become almost entirely covered with spots, for even in such cases the primary spotting—above the midlateral line, in front of and under the dorsal fin—remains apparent.

Another macromelanophore pattern of *X. v. variatus* has been designated *Sr* by Kosswig (1961), Zander (1962), and Anders & Klinke (1965) because it somewhat resembles the striped pattern *(Sr)* of *X. maculatus*. As judged by the photographs and drawings of Gordon & Smith (1938, figs. 9B, D) and Zander (1962), this pattern is best developed along the midlateral line below the dorsal fin and on the caudal peduncle. In contrast, the striped pattern of *X. maculatus* (populations from the Rio Jamapa and from British Honduras) is most evident under and in front of the dorsal fin, and is virtually absent or only weakly expressed on the caudal peduncle. Because the two patterns are phenotypically distinct, we propose to call the one from *X. v. variatus* lined *(Li)*. In Gordon & Smith (1938), there is a wild-caught male with a macromelanophore pattern that combines the features of the lined *(Li)* and punctatus *(P^1)* patterns. When crossed with a spotted *X. maculatus*, at least some of its male F^1 offspring showed the *Li* pattern alone. (All the F^1 females inherited *Sp* from *maculatus* and were melanotic.) Evidently P^1 and *Li* had segregated. Zander (1962) described six interspecific crosses in which *Li* and P^1 also segregated, and it is most probable that these are alleles.

A fourth pattern that involves spotting on the sides may very well exist, but little is known about it. Rust (1939, 1941) described some *X. variatus* that were orange along their ventral sides and in addition possessed some small spots scattered over the caudal peduncle. As judged by his photograph, this pattern is definitely different from P^1 and P^2. Rust attributed the black speckling to the gene *O* for orange (which we designate as *Or* in order to differentiate it from the *O* for one-spot), but it is almost certain that these spots resulted from a macromelanophore allele closely linked to *Or*, as Breider (1949) has suggested. That P^1 and *Or* are distinct is also shown in crosses between *X. variatus* and *X. hellerii*. When introduced into a *hellerii* genome, the punctatus pattern remains largely unchanged or only slightly modified (Rust, 1941; Zander, 1962; Anders & Klinke, 1965), while the macromelanophore allele associated with the gene *Or* is greatly increased in expressivity (Kosswig, 1948; figures 5 and 6 in Rust, 1941). It is interesting to note that in *X. variatus*, as in *X.*

[6] The *Sp* of *X. v. variatus* discussed in Gordon (1943) and Atz (1959) must include both P^1 and P^2, since these authors did not recognize that there is more than one spotted pigment pattern. Evidently, Rosen (1960, pg. 81) also did not, for he lumps all spotted patterns of this species not occurring in the Rio Cazones (from which living fish have never been brought to the laboratory) as "blotched." Nevertheless, all the spotted patterns of *X. v. variatus* treated by Atz (1962), and called *Sp* by him, actually refer to P^1, with the possible exception of the photomicrograph, fig. 18.

maculatus, a locus that controls the appearance of distinct xanthophore and erythrophore pigment patterns is apparently closely linked to the macromelanophore locus.

c. Xiphophorus milleri.

According to Rosen (1960), two macromelanophore patterns can be distinguished phenotypically in *X. milleri,* one pattern consisting of more or less definite rows of spots arranged along the dusky bands on the side, the other of irregular spots on the body. The latter pattern was present in some of the fish collected alive from Lake Catemaco in 1963, and these have been bred in the Genetics Laboratory (see Figs. 4 & 5). In our strain, the spotting occurs only in the males and consists of a fine speckling of macromelanophores mostly along the ventral half of the caudal peduncle. The melanophores are most numerous around the base of the anal fin. In some fish this area becomes solid black, and in a few fish a state of melanosis has been detected. Since this spotted ventral pattern, *Sv,* shows strictly paternal inheritance (Table II), this species presumably possesses an *XX-XY* type of sex determination with *Sv* on the *Y* chromosome (Kallman, 1965b).

d. Xiphophorus montezumae.

The macromelanophore patterns of *X. montezumae cortezi* have been described by Atz (1962). The spotted caudal pattern *(Sc)* consists typically of one or more irregular, elongated patches of heavy pigmentation commencing close to the base of the middle or lower caudal fin rays and extending posteriorly for roughly one third of the fin's length.[7] The spotted pattern *(At)* consists of numerous deeply pigmented, roundish spots, mostly confined to the mid- and post-dorsal regions above the midlateral line; as a fish grows older, spotting may also develop in its caudal and dorsal fins (Atz, 1959, 1962).[8]

Crosses involving wild-caught *X. m. cortezi* and their descendants have been summarized in Table III. The *At* gene of *cortezi* is not sex-linked and behaves as an autosomal dominant. From the 15 crosses in which a single parent possessed the *At* pattern, spotted and wild type offspring were obtained in equal frequency (89 *At* females, 88 *At* males, 94 + females, 77 + males).

TABLE II. INHERITANCE OF MACROMELANOPHORE, *Sv,* AND TAIL SPOT, *Ss, Pt,* AND *B,* PATTERNS IN *Xiphophorus milleri*

Pedigree	Parents: Female		Parents: Male	Offspring: Females					Offspring: Males							
				Sv / +	+ / +	+ / *Pt*	+ / *Ss*	+ / *PtB*	+ / *B*	*Sv* / +	*Sv* / *Pt*	*Sv* / *Ss*	*Sv* / *PtB*	*Sv* / *B*	+ / +	+ / *Pt*	+ / *Ss*	+ / *PtB* · *B*
1401	1374-½	+	1374-11	*Sv Ss (Pt)*														
1410a	1374-3	+	1374-12	*Sv B*	6				2	7				3				
1410b	1374-4	*Pt*	1374-12	*Sv B*	1	1		4	3	1	3		2	2				
1494	1410a-½	+	1410b-11	*Sv Pt B*		19			15		16			8				
1545	1410a-3	+	1401-11	*Sv Ss*	4		7			10		9						
1601	1494-½	*Pt*	1494-11	*Sv B*	3	5		3		2	2		1	1				
1628	1545-½	*Ss*	1545-11	*Sv* +	4		6			3		4						
1602	1494-3	*B*	1543-11	+	14				12						10			9
1717	1602-½	+	1628-11	*Sv Ss*	24		13			21		19						
1748	1601-½	*Pt*	1601-11	*Sv B*	2	2			5	6	3		2	2				
1784	1628-1	*Ss*	1602-11	+ *B*	6		16[1]		10						12		15[1]	4
1831	1784-1	*SsB*	1784-11	+ +			9		8								5	8
1832	1748-1	*Sv Pt*	1784-12	+ *B*	1	1			2	2	2					1		1

[1] Either *Ss* or *SsB.*

[7] Called *Nc* by Breider (1949) and Breider & Mombour (1949).

[8] *At* for atromaculatus, which, in Greek, means black spotted or dressed in black spots (Brown, 1954). This genetic factor was called *Sp* by Gordon (1943), Atz (1962), Zander (1962, 1965), and Anders & Klinke (1965).

TABLE III. INHERITANCE OF MACROMELANOPHORE PATTERNS, *At* AND *Sc*, IN *Xiphophorus montezumae cortezi*

Pedigree	Parents				Offspring									
	Pedigree		Phenotype		AtSc		Sc		At		+		Total	
Xmc-	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
21	1	11	+	*Sc*	..	..	11	13	..	..	19	13	30	26
22	2	12	+	*AtSc*	..	3	..	1	6	6	6	4	12	14
23	3	13	*At*	+	..	4	..	1	9	6	5	10	14	21
24	4	14	*At*	+	..	..	..	..	5	4	9	8	14	12
25	5	15	*At*	*At*	..	..	..	..	9	6	1	6	10	12
26	6	16	*At*	*At*	..	..	..	..	10	2	2	2	12	4
27	7	17	*At*	*Sc*	..	2	..	2	3	..	2	1	5	5
28	8	18	+	*At*	..	..	..	..	4	2	2	3	6	5
29	9	19	*At*	*At*	..	..	..	..	18	35	6	11	24	46
30	21-1	23-11	+	*AtSc*	..	1	..	1	2	4	8	..	10	6
30[2]	30-1	30-12	+	*Sc*	..	..	5	9	..	..	6	5	11	14
31	21-2	15-11	*Sc*	+	..	..	7	11	..	..	18	6	25	17
32	30-8	30[2]-12	*At*	*Sc*	4	6	4	1	1	4	3	2	12	13
33	31-8	30[2]-11	+	*At*	..	2	1	..	6	7	6	5	13	14
34	33-6	32-16	+	*AtSc*	..	1	..	1	2	5	7	4	9	11
35	33-7	32-17	+	*AtSc*	..	1	..	2	3	6	8	1	11	10
36	33-8	32-15	+	*AtSc*	2	1	..	2	6	9	5	4	13	16
37	32-2	33-11	*AtSc*	+	3	2	1	3	7	2	5	4	16	11
38	33-5	33-15	+	*At*	..	..	..	..	4	7	5	8	9	15
39	30[2]-1	30-11	*Sc*	*AtSc*	1	..	..	1	2	..	3	..	6	1
40	33-1	33-11	*At*	+	..	..	..	1	19	3	14	7	33	11
41	32-14	32-14	*AtSc*	*AtSc*	5	7	1	3	4	1	3	1	13	12
											Total		308	296

Four crosses in which both parents exhibited the *At* pattern yielded spotted and wild type offspring in a ratio of 3:1 (97 *At*, 36 +). This indicates that the *At* parents were heterozygous.

The inheritance of the spotted caudal *(Sc)* gene is difficult to study, however, because of its low penetrance. Neither of the progenitors of strain 38 exhibited the *Sc* gene, although it must have it present in at least one of them (Table IV). This strain has been inbred and maintained in the Genetics Laboratory for more than 15 generations. In the sixth generation, the fish evidently became homozygous for *At*, because from then on no more fish appeared that lacked this pattern. As reported by Atz (1962) and Zander (1965), but without any supporting data, the *At* and *Sc* genes are not allelic. During 10 generations of mating *AtSc* females with *AtSc* males, not a single fish was obtained that exhibited only the spotted caudal pattern. During the last 10 generations, 205 fish were *AtSc* and 74 were *At*. The latter are undoubtedly the result of nonpenetrance of the *Sc* gene. The spotted caudal pattern is sometimes represented by only a few macromelanophores, and a mating of two spotted fish from the 11th and 12th generations, respectively, resulted in five *At* and nine *AtSc* offspring. Crosses between wild type *X. m. montezumae* females and *AtSc* males of *cortezi* also indicate that *At* and *Sc* segregate independently, since many hybrids exhibited either *At* or *Sc* alone, or both (Table V). Two males appear to have been homozygous and two heterozygous for the *At* gene.

There is no evidence that *Sc* is sex linked. In strain 38 (Table IV), a significantly higher percentage of males showed the *Sc* pattern, but this could result from a higher penetrance in males. The sex ratio of strain 38 does not differ from 1:1; in fact, we have obtained a total of 458 males and 458 females (Tables III & IV), and Kosswig (1959) reported 112 males and 112 females. Zander (1965) has recently suggested that two types of males occur in *X. m. cortezi*. He obtained males that sired broods in which all, or nearly all, the offspring were females, and he believes that these males were *XX* fish in which sex had been determined by autosomal male factors. Other males, believed to be *XY*, sired offspring that were 50 percent or more male. Zander also stated that *XX* males are of much more frequent occurrence in *X. m. cortezi* than in *X. maculatus*, but offered no direct evidence to support this claim. Since Zander (1965)

TABLE IV. INHERITANCE OF MACROMELANOPHORE PATTERNS, *At* AND *Sc*, IN STRAIN 38 OF *Xiphophorus montezumae cortezi*

Generation	Parents		Offspring								
			+		*Sc*		*At*		*AtSc*		
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	Total
2	*Sp*[1]	*Sp*[1]	2	..	..	..	4	1	3	2	12
3	*AtSc*	*AtSc*	..	1	1	1	..	3	2	4	12
4						Not recorded					
5	*AtSc*	*AtSc*	3	2	..	1	2	..	2	1	11
6a	*AtSc*	*AtSc*					1	..	..	5	6
6b	*AtSc*	*AtSc*					3	1	2	5	11
7b	*AtSc*	*AtSc*					6	2	8	11	27
8	*AtSc*	*AtSc*					2	..	7	22	31
9b	*AtSc*	*AtSc*					1	..	1	2	4
9c	*AtSc*	*AtSc*					7	2	3	4	16
9d	*AtSc*	*AtSc*					5	2	1	1	9
10	*AtSc*	*AtSc*					1	1	13	15	30
11a	*AtSc*	*AtSc*					..	..	5	4	9
11b	*AtSc*	*AtSc*					1	7	12	17	37
12b	*AtSc*	*AtSc*					2	..	2	2	6
13a	*AtSc*	*AtSc*					8	4	3	10	25
13b	*AtSc*	*AtSc*					2	..	1	2	5
14b	*AtSc*	*AtSc*					..	2	2	5	9
14c	*AtSc*	*AtSc*					4	..	1	6	11
15a	*AtSc*	*AtSc*					2	..	3	6	11
15b	*AtSc*	*AtSc*					6	..	7	4	17
15c	*AtSc*	*AtSc*					1	..	1	3	5
15d	*AtSc*	*AtSc*					1	..	5	4	10
	Total		5	3	1	2	59	25	84	135	314
									Males:		165
									Females:		149

[1] From ped. 38

does not record the sex ratio of his stock of *X. m. cortezi* and lists only the sex ratios of a few selected crosses, without indicating how the fish are related to one another, it cannot be determined whether these crosses are representative of the species as a whole. Our sex ratio data, for example, provide no evidence for the existence of *XX* males in *X. m. cortezi*. In only two out of 44 crosses did the sex ratio differ significantly from 1:1 (ped. 29 and 40, Table III); in one there was an excess of males and in the other an excess of females, but neither deviation was as large as the ones reported by Zander.

Xanthophores and xanthoerythrophores are present in small numbers in *X. montezumae* (Öktay, 1964). Some males of *X. m. montezumae* have bright orange swords, and in both subspecies fish are found with bright yellow

TABLE V. HYBRIDS BETWEEN FEMALES OF *X. m. montezumae* AND MALES OF *X. m. cortezi*

Pedigree	Parents				Offspring							
	Female		Male		*At*		*Sc*[1]		*AtSc*[1]		+	
					♀	♂	♀	♂	♀	♂	♀	♂
900a	733-3	+	38[6]-13	*AtSc*	7	..	..	..	4	7	..	..
900b	733-4	+	38[6]-14	*AtSc*	4	1	..	..	6	12	..	..
900c	733-5	+	38[6]-15	*AtSc*	3	..	3	2	5	..	..	..
900d	733-6	+	38[6]-15	*AtSc*	3	..	4	3	7	12	1	..

[1] In five females and eight males, the *Sc* pattern is so weakly developed it can hardly be recognized.

dorsal fins. Whether this red and yellow pigmentation is genetically homologous to the polymorphism found in *X. variatus* and *X. maculatus* is not known. By means of introgressive hybridization, Myron Gordon introduced the chromosome of *cortezi* that carries the *Sc* gene into the 3B strain of *X. hellerii strigatus*. In the backcross hybrids, the *Sc* pattern developed slowly and varied greatly in its expression. In some fish the entire caudal fin and caudal peduncle ultimately turned black; in others only a few macromelanophores were present in the caudal fin at the age of one year, and in some fish the penetrance of the *Sc* gene was nil. An idea of the variability of this pattern can be gained from photographs in Atz (1962), Breider & Mombour (1949), Gordon (1956a), Marcus & Gordon (1954), and Zander (1965). Most striking in the *Sc* backcross hybrids is their red body coloration. In our laboratory no hybrid with the *Sc* pattern has ever been seen that did not possess the red pigmentation. During the 6th to 10th generations, produced by backcrossing red, spotted caudal fish with 3B swordtails, 288 offspring were obtained of which 90 were both red and spotted caudal, 53 were red, and 145 were wild type (non-red, non-*Sc*). There is little doubt that the red offspring were fish in which the *Sc* gene was not expressed even though it was present. That the sum of the red fish and the red and spotted caudal fish (143) in effect equals the number of wild type fish supports this view. In his popular account of the backcross hybrids, called the Red Jet strain because of their striking pigmentation, Gordon (1956a) indicated that the red coloration resulted from an enhancement of the red stripes of *X. hellerii* (very weakly developed in the 3B strain), as a result of modifying genes introduced from *X. montezumae cortezi*. Öktay (1964) also indicated that pigmentation of the red stripes of *X. hellerii* is increased after hybridization with *X. montezumae*, but offered no evidence. The explanation of Gordon and Öktay is difficult to reconcile with the observation that no *Sc* backcross hybrid ever appeared that lacked red pigmentation. Furthermore, in the tenth backcross generation, in which there were 16 red and *Sc*, 18 red, and 33 +) individuals, most of the chromosomes must have been derived from *hellerii*. With the reduction in the number of *cortezi* chromosomes and modifiers, a gradual return of the red pigmentation to the one typical of the pure 3B line would be expected. Instead, the intensity of the pigmentation remained more or less constant from the early to the latest generations.

In a more recent repetition of Gordon's series of crosses, a male *X. m. cortezi* (14th generation of strain 38) was mated with a female *X. h. strigatus*, a descendant of a fish collected in the Rio Sarabia (ped. 1377). Only three hybrids were obtained, all red and *Sc*. In the first backcross generation to *hellerii* (ped. 1600), 19 of the fish were red and spotted caudal and 25 were wild type. In the second backcross generation (ped. 1707), 35 were red and spotted caudal, two were red only, and 44 were wild type. Red pigmentation and the *Sc* pattern were again inherited together, just as in Gordon's series of backcrosses. In contrast to Gordon (1956a) and Öktay (1964), we suggest that the red pigmentation of the *hellerii* x *montezumae* hybrids arises from a specific gene of *m. cortezi* which is linked to *Sc*. In *X. montezumae cortezi*, the phenotypic effect of this gene, if it has one, has not yet been recognized. It is possible that it exists in more than one allelic state: one that gives rise to intense red pigmentation in hybrids with *hellerii* (the one that is present in our stock of *X. m. cortezi*) and the other with no such effect. This would account for the lack of red body pigmentation in the hybrids of Kosswig (1936) and Breider & Mombour (1949).

The existence of genes that have no visible effect is, of course, well known in this genus. The *Sc* gene is one example, although its expression is suppressed only in a certain percentage of fish (Table IV). Other examples involving macromelanophore genes are known from *X. maculatus*. Fish with macromelanophore alleles may show no visible pattern, and the presence of these genetic factors may be revealed in crosses with other populations or species (Gordon, 1951a; Kallman, 1965a). Similar observations have been made on the *Dr* gene (red dorsal) of *X. maculatus*. In hybrids with *variatus, hellerii,* and *couchianus,* not only the dorsal fin is red, but almost the entire body of the fish from the level of the dorsal fin backwards (Kosswig, 1937, 1948, 1959, 1961; Gordon, 1948, 1950b; Atz, 1962; Zander, 1962). In crosses with *montezumae* and *pygmaeus,* however, the expression of *Dr* is suppressed completely (Kosswig, 1961; Zander, 1962). Powerful genetic mechanisms are evidently present, mechanisms that hold the expression of pigment patterns within a norm in a given population or gene pool. It is not difficult to visualize how such a genetic system could evolve further so that finally the gene has no visible effect in any member of the population, although it may retain other important functions. Only when such a genetic mechanism is destroyed through out-crossing will the presence of such a gene be demonstrated.

If our interpretation of the red coloration in *X. m. cortezi—X. hellerii* hybrids is correct, *cor-*

TABLE VI. INHERITANCE OF MACROMELANOPHORE PATTERN, Db^1, IN A STRAIN OF *Xiphophorus hellerii guentheri*

Pedigree	Parents				Offspring					
	Female		Male		Db^1			+		
					♀	♂	Immature	♀	♂	Immature
	Bx-1	Db^1	Bx-12	+	16	19		15	26	
Bx^2	Bx-2	+	Bx-11	Db^1	8	4		7	11	
$Bx^{7(1)}$	Bx^6-1	Db^1	Bx^6-11	Db^1	8	33		3	5	
$Bx^{8-17(2)}$	Bx	Db^1	Bx	Db^1	170	292	10			
321a	$3B^9$-1	+	Bx-11	Db^1	18	20		35	23	
321b	$3B^9$-2	+	Bx-14	Db^1	7	9		12	11	
323	Cx-2	+	Bx-13	Db^1	8	7		12	9	
481[3]	Bx	Db^1	Hx-11	Db^2	6	12		5	5	
488	Bx^2-2	Db^1	Hx-13	+	10	11	24	4	6	24

(1) Offspring of third through sixth generation not recorded.
(2) Summary of 15 crosses involving 10 generations.
(3) Eleven of the 18 fish that were examined exhibited Db^2.

tezi represents the third species in which a gene controlling pigmentation by erythrophores is linked to a macromelanophore locus.

e. Xiphophorus hellerii.

In several populations of *X. h. guentheri* and *X. h. strigatus,* a small proportion of fish exhibit macromelanophore spotting (Rosen, 1960, pg. 120, 125, 126). The pattern that is present in the Bx strain has been described by Atz (1962). This pattern (Db^1) is dominant, autosomal, and its penetrance appears to be 100% (Table VI; Fig. 9).[9] The two wild-caught spotted fish were undoubtedly heterozygous for this allele, since when they were mated to wild type individuals, both spotted and non-spotted fish appeared in equal frequency among the offspring. A mating of two spotted fish with each other gave rise to 41 spotted and eight non-spotted individuals, a ratio that does not differ significantly from the expected 3:1. Inbreeding Db^1 fish brother-to-sister for 10 generations has produced the Bx strain. Fifteen crosses resulted in 170 females and 292 males, all spotted. When a spotted swordtail of the inbred Bx line was outcrossed to a wild type *X. hellerii,* the offspring (14 females, 17 males) were all spotted. The Bx strain must be homozygous (Db^1Db^1).

A second stock of swordtails with a spotted pattern can be traced to fish collected by Myron Gordon in the Rio Lancetilla, Honduras (see Fig. 10). This stock, Hx, was bred in our laboratory for only four generations, but its descendants are still maintained in the laboratory of Dr. Curt Kosswig in Germany. The pattern in the Hx strain is controlled by an autosomal dominant gene that shows 100% penetrance in the Hx stock and in F_1 hybrids with other strains of swordtails (Table VII). In the Hx fish, as pointed out by Rosen (1960, p. 126), the spots show a tendency to be arranged in rows, especially in older specimens. Fish with this more or less striped pattern have been illustrated by Zander (1962, Table III, Fig. 3) and Peters (1964, Fig. 5). Only 20 of our Hx fish have been preserved and of these, 19 have the striped arrangement of macromelanophore spots on at least some part of their flanks. This pattern is clearly different from the spotting of the Bx strain. Of 55 Bx fish examined, only 10 had small stripes, and these were irregular and never included more than four spots, thus being unlike the Hx stripes which in many cases are composed of eight to ten spots. Several crosses suggest that the difference in the spotted patterns of the Bx and Hx lines do not result from modifying factors, but from different genes. In pedigree 488 (Table VI) in which the Db^1 gene was introduced by the Bx line, none of the spotted offspring showed stripes, whereas in pedigree 481 in which both parents introduced genes for spotting 11 of the 18 fish examined showed a striped pattern. We have also examined 31 available F_1 hybrids of Gx x Hx and Cd x Hx, and 18 of these had spots arranged in horizontal rows. The remaining 13 fish were small and weakly spotted. In contrast, only two of 41 available F_1 hybrids of Cx x Bx or 3B x Bx showed any tendency towards an arrangement of their

[9] Designated Db^1 for dabbed, which refers to the irregular size and shape of the spots (Atz, 1962). This factor was called *Sp* by Atz (1962). The evidence for incomplete penetrance mentioned by this author does not exist.

TABLE VII. INHERITANCE OF MACROMELANOPHORE PATTERN, Db^2, IN A STRAIN OF *Xiphophorus hellerii guentheri*

Pedigree	Parents				Offspring					
	Female		Male		Db^2			+		
					♀	♂	Immature	♀	♂	Immature
	Hx-1	+	Hx-11	Db^2	8	5	21	13	5	13
Hx^2	Hx-2	+	Hx-12	Db^2	14	14	14	15	8	13
Hx^3-a	Hx^2-1	Db^2	Hx^2-11	Db^2	3	4		1	3	
Hx^3-b	Hx^2-2	Db^2	Hx^2-12	Db^2		7		3	3	
422	Cd^2-4	+	Hx-14	Db^2	11	8		7	13	
824	Gx	+	Hx^4-11	Db^2	10	14	1			
825	$3B^{16}$	+	Hx^4-11	Db^2			28			
481(1)	Bx	Db^1	Hx-11	Db^2	6	12		5	5	
482	481-1	*Db*	481-11	*Db*	11	9	57	3	4	16
483	482-2	*Db*	481-12	*Db*	7	10		1	4	

(1) In pedigrees 481, 482, and 483, Db^1 and Db^2 were not distinguished. These crosses indicate that both patterns are inherited as autosomal dominants.

spots in stripes. The macromelanophore patterns of the Bx and Hx strains show consistent differences that are maintained in hybrids. In our opinion, they are caused by different genes, which we designate Db^1 and Db^2, although no critical experiment has yet been performed to determine whether or not they are alleles.

The problems that can arise with a *Xiphophorus* of indefinite ancestry or uncertain geographic origin are well illustrated by the history of the montezuma *(Mo)* factor. Kosswig was the first to show that the orange or orange-red coloration and the numerous black spots of the so-called montezuma swordtail were inherited together and behaved as if controlled by an autosomal dominant gene, which he called *Mo*. Following the views of a leading aquarist, Christian Brüning, Kosswig (1933, 1934) believed that this factor had originated from a pair of *X. montezumae* that had been imported into Germany just before the first World War and that it had been perpetuated by twenty years of backcrossing to aquarium stocks of *X. hellerii*. When the first living *X. montezumae cortezi* were imported, however, it was apparent that the pigmentation of this species bore no resemblance to the montezuma pattern (Gordon, 1938), and Kosswig (1935b, 1937) then suggested that *Mo* was most likely a gene belonging to *X. hellerii*. Breider (1936) reported that a fish with pigmentation identical to *Mo* had appeared among the offspring of a mating between a wild type and a red swordtail. He supposed this to be a mutant but was unable to test it because the fish was sterile. Nevertheless, Breider (1936) and Kosswig (1936) both recognized the possibility that some other species of *Xiphophorus* might have been the source of *Mo*.[10] Gordon (1943, 1948) found he could reproduce the montezuma pattern by hybridizing *X. maculatus* that carried the factors for striped and red dorsal *(Sr Dr)*, with *X. hellerii* and then backcrossing the F_1 to *hellerii*. He concluded that *Mo* is "probably homologous" with *Sr* and *Dr* and that the montezuma variety of swordtail was of hybrid origin. Breider (1949), Kosswig & Öktay (1955), and Öktay (1964) agreed with this view. There seems to be no question of the hybrid origin of the so-called montezuma swordtail, especially when it is noted that no specimens with *Mo*, or any pigmentation at all like it, have ever found among the thousands caught in nature. The *Sr* and *Dr* must have been closely linked in the platyfish progenitor of the montezuma variety—as they are known to be in the strain Jp 30 in our laboratory.

At least two other genes for color patterns of unknown origin have been recorded in *X. hellerii, viz.* seminigra *(Sn)* and rubescens *(Rb)*,

[10] Kosswig & Sengün (1945) suggested that the species described by Ahl (1938) as *Xiphophorus pseudomontezumae* was most probably the form from which *Mo* was introduced into *X. hellerii* through hybridization. Breider (1938) had previously indicated that this fish, which was then still undescribed, might have been the source of *Mo*. Indeed, the specimens were undoubtedly among the first so-called *X. montezumae* that turned up in Germany before the first World War (Ahl, 1938). The two specimens upon which Ahl based his description give every evidence of being hybrids, however, most likely between *X. maculatus* and *X. hellerii*, but possibly between *X. v. variatus* and *X. hellerii*. Ahl gave the type locality simply as Mexico, no more exact information being available. We are convinced that some home aquarium was the real place of origin.

the former characterized by black pigmentation on the lower half of the body and the latter by a red coloration that commences at the base of the tail and covers most of the body (Breider, 1938; Kosswig, 1939). As with the montezuma *(Mo)* pattern, no wild swordtail with pigmentation resembling *Sn* or *Rb* has ever been seen. The hybrids between *X. maculatus* with red coloration and *X. hellerii* show an extension and intensification of the red, and this effect becomes more pronounced in backcrosses to *hellerii*. Undoubtedly the red swordtails of commerce owe their color to genes of *X. maculatus* that have been introgressively incorporated into their genomes (Gordon, 1943, 1946b, 1948). Kosswig (1961) and Öktay (1964) came to the conclusion that *Rb* was a *maculatus* gene introduced into domesticated swordtails.

2. The Micromelanophore Tail Patterns.

a. Xiphophorus maculatus.

The morphology, genetics, and geography of the tail patterns of *X. maculatus* have been studied by Gordon (1931, 1937b, 1946b, 1947b, 1956b), Gordon & Fraser (1931), Gordon & Gordon (1950, 1957), and Kerrigan (1934). Gordon recognized seven basic pigment patterns in addition to the unmarked wild type, and he showed that they were members of a single autosomal, dominant allelic series (Gordon & Fraser, 1931; Gordon, 1947b). Up to the present, no fish with more than two of these patterns has been recorded, either from nature or the laboratory (Rosen, 1960, pg. 76). Whether four other tail patterns that are rarely seen—upper and lower comet, axhead, and cut-crescent—also belong to this allelic series is not known.

New evidence indicates there are two distinct tail patterns that were previously lumped under the category of "one-spot". One of these patterns, *O*, is present in homozygous condition in the Jp 30 strain and is identical in its morphology with the pattern described as one-spot by Gordon (1931) (see Fig. 6). Several photographs of fish possessing this pigmentation have been published (Gordon, 1947a; Gordon, 1951b, the male in fig. 4; Gordon, 1952, fig. 2, plate 1; Gordon & Gordon, 1957, the female in fig. 4, plate 2; Sterba, 1963, the female in fig. 760). A distinctly different pattern, which we call dot *(D)* because of its small size, is present in the A and B lines of Jp 163, both of which are homozygous for it. Photographs of fish possessing dot may be found in Gordon & Gordon (1957, the females in plate 1, figs. 1 and 3), Kallman & Gordon (1958), MacIntyre (1961b), and Sterba (1963, the male in figs. 760 and 761). Differences between the two patterns are readily apparent even in newborn fish. One-spot is then visible as a small black area, while dot does not develop until later on. In specimens six months old or older,. the one-spot pattern covers the entire hyplural bone except for its most anterior apex. As Gordon (1931) pointed out, the posterior margin of this pattern coincides with the part of the hyplural bone with which the caudal fin rays articulate. Although the dot pattern occupies a similar position and shows considerable variation, it is always less than half the size of the one-spot. It is slightly irregular in outline and consists typically of two narrow, intensely black lines of pigment cells that occur just above and below the horizontal septum, which divides the fish along the midlateral line into dorsal and ventral halves. These bands of pigment cells run anteriorly approximately one half the length of the hyplural bone. The posterior margin of the dot coincides with that of the one-spot. A narrow band of pigment cells is often present just anterior to the articulation of the caudal fin rays with the hyplural bone in dot, but the pigment cells do not extend as far dorsally or ventrally as in one-spot. Especially in older, more heavily pigmented fish, pigment cells may completely fill in the angles formed by the horizontal and vertical components of the dot pattern, giving it a somewhat triangular appearance. In small fish, the one-spot pattern may be similar in size to the dot in larger fish, but the difference in shape is always present. Fish that are heterozygous for the two patterns look like those with one-spot alone.

Both patterns have been called "one-spot" in the past and treated as if they were caused by the same allele.[11] If this is true, the difference in phenotypic expression must be the result of modifying genes. But when fish belonging either to strain Jp 30 or Jp 163 were outcrossed to other stocks of *X. maculatus* or to other species, the integrity of the dot and the one-spot patterns was maintained in every case. Of the many crosses that demonstrate one-spot and dot to be controlled by different alleles, two are described here. In the first backcross generation of (Jp 30 x *X. couchianus*) x *X. couchianus*, all fish that inherited the tail spot pattern from the Jp 30 strain were typically one-spot in appearance, although the overall intensity of the pigmentation was much greater than in the pure species (Table VIII, ped. 1095, 1161, 1166). On the other hand, in the first and second backcross generations of (Jp 163 x *X. couchianus*) x *X. couchianus*, all fish with the tail spot pattern possessed

[11]For example, by Gordon (1943, 1947b), Rosen (1960), and Atz (1959).

TABLE VIII. INHERITANCE OF TAIL SPOT PATTERNS IN *Xiphophorus maculatus* (PHENOTYPES ONLY INDICATED)

Pedigree	Parents				Offspring						
	Female		Male		*O*	*D*	*Cc*	*Cc O*	*Cc D*	+	*DT*
1095	C-30	*O*	Xc-G	+	16						
1161	1095-½	*O*	Xc-G	+	33					45	
1166	Xc-G	+	1095-11	*O*	6					4	
845	Jp 163B	*D*	Xc-G	+		34					
881	845-½	*D*	Xc-G	+		14				9	
945, 946	881-½	*D*	Xc-G	+		7				8	
270	Jp-30	*O*	Cp-11	*Cc D*				100			
270[2]	270-2	*Cc O*	270-12	*Cc O*	12			21	11		
292	270-1	*Cc O*	Cp-11	*Cc D*				44	50		
1380a, b	1342-1, 2	*Cc D*	Jp 163B	*D*		48			46		
1500	1380a-1	*Cc D*	Cp	+		102			99		
1923	1900-21	*Cc O*	Hp-2	*T*				26			30

the dot pattern, although the expression of the *D* allele was also enhanced in the hybrids (Table VIII, ped. 845, 881, 945, 946; Fig. 2).

Three apparent exceptions to the rule that no platyfish can inherit or possess more than two tail patterns (as is required for true allellism) have been encountered. The first case involved a male platyfish (Cp-11), collected in 1948 in the Rio Coatzacoalcos. This male, which had the complete-crescent and dot patterns, was mated to a female of strain Jp 30 homozygous for one-spot (see Fig. 6). All of the 100 offspring exhibited the one-spot and the complete-crescent patterns (Table VIII, ped. 270; Fig. 7). Despite its phenotype, the male parent must have been homozygous for the *Cc* allele. When one of the F_1 females was backcrossed to Cp-11, one half of the offspring exhibited the one-spot and complete-crescent patterns and the other half the dot and complete-crescent (ped. 292). When two F_1 fish were mated, 50% of the F_2 were complete-crescent and one-spot, and 25% were complete-crescent and dot, and 25% were one-spot (ped. 270[2]). These results can be explained by recalling that one-spot, dot fish are phenotypically identical with one-spot fish and by assuming that dot and complete-crescent were inherited together:

CcD Cc (male) x O O (female)

O CcD complete-crescent, one-spot (50% F_1)
O Cc complete-crescent, one-spot (50% F_1)

O CcD x O CcD

O O one-spot (25% F_2)
O CcD complete-crescent, one-spot (50% F_2)
CcD CcD complete-crescent, dot (25% F_2)

A similar case was discovered in 1963. Five fish that had been collected in the Rio San Pedro at Carmelita, Guatemala, exhibited both *Cc* and *D*. Two females were mated to Jp 163 B *(DD)* males in order to determine the constitution of their sex chromosomes (Kallman, 1965a). As expected, all the offspring (Table VIII, ped. 1380) showed dot and one half of the fish showed complete-crescent. One F_1 female *(CcD)* was mated to a male of the Cp (Coatzacoalcos) strain, which does not possess any tail spot patterns. One half of the offspring of this cross inherited dot, the other half complete-crescent and dot (ped. 1500). Again, the results can only be explained by the assumption that in the fish from Carmelita, *Cc* and *D* are inherited together. The detection of complete-crescent linked to dot is difficult because the same pattern results when *Cc* and *D* are on different chromosomes and when dot is masked by the one-spot or moon patterns. The pattern complete-crescent without dot, as described by Gordon (1931), is present in the homozygous condition in the Hp–1 line (see Fig. 8). When fish of this strain are outcrossed to other platyfish stocks, the offspring show *Cc* but never *D*, unless the latter is introduced from the other stocks.

The third case came to light recently and involved the inheritance together of one-spot and complete-crescent. A female from British Honduras (ped. 1900-21), that was phenotypically one-spot and complete-crescent, was mated with a male of the Hp-2 stock, homozygous for twin-spot *(TT)*. They produced 30 offspring that were dot and twin-spot *(DT)* and 26 that were one-spot and complete-crescent. Since complete-crescent masks twin-spot, the latter had *T* as

well as *O* and *Cc*. The genotype of the female parent undoubtedly was *OCc D*.

At the present time, it is not possible to decide whether we have identified two new alleles or whether we are dealing with a super-gene.

b. Xiphophorus variatus.

The unitary tail spot pattern *(Ps)* of *X. variatus* is morphologically and anatomically distinct from the one-spot and moon patterns of *X. maculatus*.[12] In *X. v. xiphidium* it varies in appearance from what superficially appears to be a small spot to a large blotch that occupies a considerable portion of the posterior end of the caudal peduncle (see Fig. 12). In some fish it is bounded anteriorly by the hemal and neural spines of the fourth caudal vertebra, which in this species, is the most anterior one whose spines articulate with caudal fin rays. In other individuals, the peduncular spot may extend only as far forward as the spines of the third caudal vertebra or perhaps not this far. Posteriorly, it extends well into the muscles lying between the lepidotrichia of the caudal fin, especially along the proximal portions of all bifurcated fin rays and one or two of the single ones immediately above or below them. In the caudal fin, the melanophores of this pattern occur in the muscle fascia and around blood vessels that are located between the lepidotrichia. In the caudal peduncle, the pigment cells are heavily concentrated in the fascia of the deep-lying muscles, around blood vessels and nerves. This arrangement of melanophores differs fundamentally from that of the moon and one-spot of *maculatus* in which the melanophores are primarily located in the lower dermis and the muscle fascia immediately below (see Figs. 14-17).

The peduncular spot *(Ps)* of *X. v. variatus* is very similar to the pattern of the same name in *X. v. xiphidium* but, at least according to the samples available to us, there seems to be a minor, but consistent, difference in that the pigmentation is less intense. The maximum size attained appears to be the same, but there are smaller peduncular spots in our samples of *X. v. variatus* than in any *X. v. xiphidium* that we have seen.

The crescent pattern *(C)* of both *X. v. variatus* and *X. v. xiphidium* is identical in shape and structure with the pattern of the same name found in *X. maculatus*. The anterior margin is bounded by the principal caudal blood vessel which bridges the caudal fin rays slightly posterior to their point of articulation. The posterior edge of the musculature of the caudal fin forms the posterior end of this pattern. Usually all but the most dorsal and ventral fin rays are involved. The pigment cells are primarily located in the inter-radial tissue, most heavily around the fin rays themselves. In both subspecies, the intensity of the pigmentation of the crescent may vary considerably. In some fish the pattern is jet black and the area behind its posterior margin is free from micromelanophores, thus setting off the crescent dramatically from the adjacent tissue (see Fig. 1).

The cut-crescent pattern *(Ct)* of *X. v. xiphidium* and *X. v. variatus* occupies the same area as the upper and lower parts of the crescent.[13] The dorsal part of the cut-crescent covers from six to nine caudal fin rays, usually beginning with the first (uppermost) bifurcated ray and seldom failing to include more than the most dorsal simple ray. The ventral part covers from five to nine caudal fin rays, usually beginning with the second or third simple ray, counting down from the lowermost bifurcated ray. It may include all but the lowermost one to three simple rays. The cut-crescent pattern does not differ as much in the intensity of its pigmentation as does the crescent, although the area behind the cut-crescent may also be free from micromelanophores. Fish of genotype *C Ct* can easily be distinguished from *C* or *CC* fish, since the dorsal and ventral portions of the combined patterns are much darker than the center, even when the crescent pattern is rather dusky. The anterior and posterior borders of the cut-crescent are also much less uniform and regular than those of the crescent. Frequently, there are extensions of pigmentation toward the rear around each caudal fin ray, and one, two, or three of these may reach considerably past what would be the posterior limit of the crescent pattern. This may be clearly shown when cut-crescent and crescent occur together in the same fish. Anteriorly, there may be extensions of pigmentation, but these are not as prominent as the posterior ones, although they are occasionally represented by numerous pigment cells in the deep-lying muscles of the area.

The upper (dorsal) part of the cut-crescent is frequently noticeably better expressed, that is, more intensely pigmented and somewhat larger, than the ventral one, and this tendency might be

[12] Designated *Ps* for peduncular spot. Formerly called moon (*M*) by Gordon (1943) and one-spot (*O*) by Atz (1959) and Rosen (1960).

[13] Zander (1962) calls this pattern twin-spot (*T*). Anders & Klinke (1965) provide a photograph of a *Platypoecilus xiphidium* (= *X. v. xiphidium*) with cut-crescent, which they also call twin-spot.

TABLE IX. INHERITANCE OF TAIL SPOT PATTERNS IN *Xiphophorus variatus xiphidium* (PHENOTYPES ONLY INDICATED)

Pedigree	Parents				Offspring											
	Female		Male		+		*C*		*Cu*		*Ct*		*Ps*		*C Cu*	
Px-					♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
20	Px-1	*C*	Px-11	+	22	27	3	13	..	..	..	..	..	..	..	..
21	Px-2	+	Px-12	*Ct*	12	17	..	..	5	17	..	..	..	..	..	..
22	Px-3	+	Px-13	*Ct*	4	1	..	..	..	..	3	4	..	..	..	..
23	Px-5	+	Px-14	*C*	20	16	14	5	..	..	..	..	..	..	..	..
25	23-2	*C*	21-11	+	5	8	4	6	..	..	..	..	..	..	..	..
26	23-3	*C*	21-12	*Cu*	4	4	6	3	6	2	..	..	..	..	9	2
27	23-4	*C*	21-13	+	7	2	2	2	..	..	..	..	..	..	..	..
35	26-1	*C Cu*	25-11	+	..	..	4	3	6	4	..	..	..	..	..	..
37	26-5	*C Cu*	26-11	+	..	..	12	12	12	14	..	..	..	..	..	..
43	26-6	*Cu*	27-11	+	2	3	..	..	4	5	..	..	..	..	..	..
36	26-4	*C Cu*	26-11	*Cu*	..	..	4	..	6[1]	8[1]	..	..	..	..	3[1]	5[1]
44	26-7	*Cu*	25-14	+	6	2	..	..	..	..	4	4	..	..	..	..
45	36-1	*Cu*[1]	36-11	*Cu*[1]	..	..	..	..	2	1	6	7	..	..	..	..
46	36-2	*Cu*[1]	25-15	+	..	..	..	..	13	9	..	..	..	..	..	..
50	36-4	*Cu*[1]	40-11	*C*	..	..	..	..	8	13	..	..	..	..	12	18
1251	1184-1	*PsC*	1228-12	+	..	..	13	13	..	..	..	..	14	12	..	..

[1] Fish with a strongly developed upper crescent and a very weakly developed lower crescent.

thought to find its ultimate expression in individuals that appear to lack the ventral part entirely. Close examination, however, has always revealed slight traces of the lower element. This might indicate that the cut-crescent and upper cut-crescent patterns are controlled by the same allele and represent the phenotypic expression of modifiers. It is possible, however, that the two patterns are controlled by two different alleles (*Ct* and *Cu*, respectively), because phenotypically a particular fish can almost always be assigned to one category or the other, the separation between them being marked even though the upper cut-crescent pattern usually includes a small ventral component.

In one series of experiments involving the tail spot patterns of *X. v. xiphidium*, there are indications that upper cut-crescent and cut-crescent are controlled by the same allele (Table IX). For example, a wild-caught male (Px-12) exhibited the cut-crescent pattern, yet among its descendants both cut-crescent and upper cut-crescent fish appeared. The existence of genetic factors that influnce the phenotypic expression of cut-crescent *(Ct)* is indicated by the appearance of certain hybrids. In a cross, involving a female *X. v. xiphidium* with a normally expressed cut-crescent and a male *X. v. variatus* with no tail pattern, the intraspecific hybrids exhibited tail patterns that ranged from cut-crescent to crescent (Atz, 1962). In a cross, involving a male *X. v. xiphidium* with cut-crescent and a female *X. hellerii,* the interspecific hybrids exhibited crescent tail patterns instead of cut-crescent (Zander, 1962).

c. Xiphophorus milleri

In this species three tail spot patterns are known (see Figs. 4 & 5). Bar *(B)* is composed of a diffuse, slightly crescent-shaped, narrow band of melanophores located just in front of the caudal blood vessel and the point where the caudal fin rays articulate with the axial skeleton. Its position, therefore, is quite different from that of the crescent pattern in *maculatus* and *variatus,* which is located behind the caudal blood vessel. Moreover, the melanophores of bar are primarily located in the dermis while those of crescent are found mainly around the dorsal and ventral edge of each lepidotrich. The upper and lower limits of the bar pattern are rather indistinct even when it is well developed. It does not extend as far as the middorsal or midventral lines, but usually ends at the level of the 4th and 5th caudal fin rays. Bar often does not make its appearance until several weeks after sexual maturity has been attained.

Another tail spot pattern of *X. milleri* is point *(Pt),* an intensely black pigment spot with a slightly irregular outline, located over the hyplural bone and occupying the same position as dot in *maculatus.* The diameter of *Pt* is roughly 12-15% the straight-line distance between the middorsal and midventral lines. In fish

exhibiting both *Pt* and *B*, the point pattern is visible in the center of the bar.

The third pattern is a single spot *(Ss)*, which is located in the same position as the one-spot of *maculatus*. Single spot is twice as large as point and covers roughly one third to one half of the distance between the middorsal and midventral lines. As in the case of bar, the melanophores of single spot and point are found in the lower dermis and superficial musculature and are located anterior to the point where the fin rays articulate with the caudal skeleton. Fish that carry both the *Ss* and *Pt* alleles are single spot in appearance. Breeding experiments show that all three patterns of *X. milleri* belong to a single dominant, autosomal allelic series (Table II).

d. Xiphophorus montezumae and *X. pygmaeus nigrensis*

In both subspecies of *X. montezumae*, a single highly variable tail spot pattern has been recognized, the caudal blotch *(Cb)*. An almost identical pattern occurs in *X. pygmaeus nigrensis* (see Fig. 3 and the lower male in Fig. 14 of Rosen, 1960). In all three forms, the caudal blotch varies both in shape and intensity. In addition, the melanophores of this pattern appear to be under nervous control to a much greater degree than the pigment cells of the other patterns, since the caudal blotch may disappear and reappear within a relatively short period of time, and anesthetization (with MS 222) of the fish leads to its intensification. In many fish it is well developed in the midportion of the tail only, often not extending dorso-ventrally as far as the uppermost or lowermost bifurcated caudal fin rays. A few fish have been seen in which the pattern was best developed along its posterior margin, where a narrow band of pigment cells ran dorsally and ventrally from the central black area, roughly parallel to the posterior edge of the caudal fin musculature. In some fish of both species, however, the caudal blotch is strongly developed and extends dorso-ventrally as far as the third simple caudal fin ray, and the pattern then superficially resembles the crescent pattern (see Fig. 3). The two patterns are morphologically distinct, however. The melanophores that comprise the caudal blotch are concentrated in the dermis between the musculature and the scales, in the scale pockets, and in the connective tissue fascia that run at right angles from the dermis to the upper and lower edge of each lepidotrich (see Fig. 13). Anteriorly, the caudal blotch borders the caudal blood vessel and posteriorly it extends somewhat beyond the limit of the musculature of the caudal fin.

e. Xiphophorus hellerii

Although many thousands of swordtails have been collected in many different localities comprising all the major river systems in which the species occurs, only the fish of the Rio Chajmaic, Guatemala, possess black patterns in their caudal fins (see Fig. 11). Every swordtail collected in this isolated population possessed a slightly elongated pigment spot in the ventral part of the caudal fin (Kallman, 1963). This pattern resembles the diacritical mark, grave, and it typically involves the first through the fifth ventral bifurcated caudal fin rays, although in a few fish, the first or fifth ray may not always be included in the spot. The pigment cells that make up the grave *(Gr)* pattern are located in the connective tissue and perymysium that surround the fin rays. Grave reaches its greatest posterior extension along the second (rarely the first) bifurcated caudal fin ray, and on each of the fin rays above this, the pigmentation ends progressively more anteriorly. On the fourth or fifth ray, for example, the pigmentation may consist merely of a tiny group of melanophores immediately behind the caudal vessel, with no measurable posterior extension. The anterior limit of the pattern is along the major caudal blood vessel, although a few pigment cells may also be found between this vessel and the point of articulation of the fin rays with the axial skeleton. The pattern makes its first appearance in fish that are two to three weeks old. In males, grave eventually becomes the black dorsal margin of the caudal sword. Swordtails from the Rio Chajmaic were established in the Genetics Laboratory (Ch strain) in 1963 and are now in the fourth generation. Seven matings have produced 22 males and 205 females, all of which exhibited this pigment pattern.

3. Chromosome Homology.

a. *Chromosomes with Macromelanophore Patterns.*

The sex chromosomes of *X. v. variatus*, *X. v. xiphidium*, and *X. maculatus* are homologous with one another. In one cross, a female hybrid of *variatus* x *xiphidium* (h-2), carrying the P^1 of *variatus* and Fl^1 of *xiphidium* was crossed with a wild type *couchianus* x *maculatus* hybrid. Eleven offspring (h-28) inherited P^1 and eleven others inherited Fl^1. None of the offspring was wild type or exhibited both patterns. In a second cross, a male F_1 *maculatus* x *v. variatus* hybrid, with the *Sp* of *maculatus* on its *X* chromosome and the P^1 of *variatus* on its *Y*, was backcrossed to a wild type *variatus*. The offspring (h-61) consisted of 32 *Sp* females, one *Sp* male, 40 P^1 males, one P^1 female, and one wild type male

(Atz, 1962, figs. 4-6).[14] The exceptional wild type male was not used in further matings, but it was probably the result of non-expression of the P^1 gene. The offspring with the P^1 pattern were sparsely marked; *e.g.*, one punctatus offspring possessed only six macromelanophores on one side and none on the other. A third cross, reported by Öktay (1962), also demonstrates that the *X* chromosome of *maculatus* and the *Y* chromosome of *xiphidium* behave as homologous chromosomes.

The sex chromosomes of *maculatus* are also homologous with those of *milleri*. When an F_1 hybrid of *maculatus* x *milleri*, carrying the *X* chromosome of *maculatus* marked either by *Sp* or *Sd* and the *Y* chromosome of *milleri* marked by *Sv*, was backcrossed to a wild type female of *X. milleri*, the offspring inherited either the macromelanophore gene of *maculatus* or *milleri*, but never both of them or neither one (Table X).

Breider & Mombour (1949) reported crosses between *X. montezumae cortezi* with the *Sc* pattern and *X. hellerii* obtained from a commercial source and of unknown history. One of their *hellerii* had a striking red body pigmentation that Breider & Mombour attributed to the gene *Rb* (rubescens) of the swordtail. No such gene is known to be present in this species, however, and it must have been introduced into their swordtail stock through prior hybridization with some other *Xiphophorus*, most likely *X. maculatus*. Most interesting is that when one of the *Sc Rb* hybrids was mated to a wild type swordtail, the *Sc* and *Rb* genes segregated. Forty of the offspring were red and thirty-one were spotted caudal. According to these results, the *Sc* gene of *X. m. cortezi* is located on a chromosome homologous to one of another species of *Xiphophorus*, carrying *Rb*. If, indeed, it should turn out that *Rb* is a gene from *X. maculatus* and is a member of the sex-linked multiple allelic series governing erythrophore and xanthophore pigmentation, the experiment of Breider & Mombour would indicate that the chromosome of *X. montezumae cortezi* carrying *Sc* is homologous to the sex chromosome of *X. maculatus*. This cross should be repeated with fish of known ancestry.

The chromosome of *X. hellerii guentheri* (Bx strain) that carries the macromelanophore allele Db^1 is not homologous with the sex chromosomes of *X. maculatus* (Gordon, 1958). When hybrids possessing the Db^1 of *hellerii* and a macromelanophore gene of *X. maculatus* were crossed to wild type fish, four classes of offspring were obtained: 67 were wild type, 74 showed the spots from *hellerii*, 37 exhibited only the *maculatus* macromelanophore pattern, and 28 fish possessed the pigment pattern of both species (Table XI). There is also evidence that the Db^1 of *hellerii* and the tail spot locus of *X. maculatus* are not located on homologous chromosomes (Table XI).

b. *Tail Spot Patterns.*

Two crosses suggest that the loci for tail patterns of *X. maculatus* and *X. v. xiphidium* are located on homologous chromosomes. A female F_1 hybrid of *X. maculatus* x *X. v. xiphidium* (h-20) possessing the comet pattern *(Co)* of *maculatus* and the crescent *(C)* of *xiphidium* was mated to a wild type male of the latter species. The backcross generation (h-30) consisted of 30 *Co*, 5 *C* and 4 wild type offspring. In a similar second cross, a male F_1 hybrid between *X. maculatus* and *X. v. xiphidium* (h-20) carrying *Co C* was mated with a female hybrid between *X. v. variatus* and *X. v. xiphidium* that had no tail patterns. The offspring (h-38) consisted of 57 *Co*, 16 *C* and 8 wild type fish. Since no fish that exhibited both the *Co* and *C* patterns appeared among the offspring of the two crosses, we conclude that the tail spot locus of *X. v. xiphidium* is located on a chromosome homologous to the one carrying the tail spot locus of *X. maculatus*. The 12 exceptional wild type fish may be explained by the late development of the

[14] The numbers given by Atz (1962) in the caption for fig. 6 are incorrect.

TABLE X. TESTS FOR HOMOLOGY OF SEX CHROMOSOMES OF *Xiphophorus maculatus* (Gp) AND *X. milleri*

Pedigree	Parents						Offspring					
	Female			Male			Female			Male		
							Sp	*Sd*	*Sv*	*Sv*	*Sp Sv*	*Sd Sv*
1781	Gp	X_{Sp}	X_{Sd}	1717-11	X_+	Y_{Sv}	25	36	..	..	35	35
1858	1748-4/5	X_+	X_+	1781-11	X_{Sp}	Y_{Sv}	37	..	1	31	..	..
1863	1748-6/7	X_+	X_+	1781-12	X_{Sd}	Y_{Sv}	..	31	..	45	..	..

TABLE XI. TESTS FOR ALLELISM OF Db^1 OF *Xiphophorus hellerii* (Bx) AND *Sd*, *Sr*, AND *O* OF *X. maculatus*

Pedigree	Parents: Female	Parents: Male	Offspring: Db^1 *Sd*	Offspring: Db^1 *Sr*	Offspring: Db^1	Offspring: *Sr*	Offspring: +
			+ +	*Cc* + *O*	*Cc* + *O*	*Cc* + *O*	*Cc* +
486	384-1[1] Db^1 *Sr O*	Gp + +	.. ..	6 .. 2	5 .. 6	4 .. 2	15 .. 9
421	385-1[1] Db^1 *Sr O*	Gp + +	.. ..	5 .. 2	27 .. 22	14 .. 10	21 .. 11
458	Gx + +	421-11[2] Db^1 *Sr O Cc*	.. ..	3 2 ..	.. 3 ..	1 1 ..	3 1 ..
672	479-1[3] Db^1 *Sd* + +	3B + +	3 1	.. .. ..	 3	.. .. ..	 2
671	Gx + +	479-11[3] Db^1 *Sd* +	5 4	.. .. ..	 8	.. .. ..	 5

[1] (Bx x *X. maculatus*) x *X. maculatus*.
[2] [(Bx x *X. maculatus*) x *X. maculatus*] x *X. maculatus*.
[3] Bx x (*X. maculatus* x 3B).

TABLE XII. INHERITANCE OF SEX AND PIGMENT PATTERNS IN F_1 HYBRIDS OF *Xiphophorus maculatus* AND *X. milleri*

P_1	
X. milleri ♀	*X. maculatus* ♂
1401	Hp-2
X_+X_+Ss +	X_+Y_{Sd} *T T*

F_1 (ped. 1532)

X_+Y_{Sd} *T Ss*	(23 ♂♂)
X_+X_+ *T Ss*	(7 ♀♀, 11 ♂♂)
X_+Y_{Sd} *T* +	(19 ♂♂)
X_+X_+ *T* +	(6 ♀♀, 9 ♂♂)

crescent pattern, since many of these fish were sacrificed soon after they had reached sexual maturity. Even if these 12 fish were added to the *C* offspring, however, there would still remain a large unexplained excess of *Co* individuals.

There is more conclusive evidence that the genes for the tail spot patterns of *X. maculatus* and *X. milleri* are located on homologous chromosomes. A female *X. milleri*, heterozygous for single spot *(Ss)*, was mated to a male of *X. maculatus*, homozygous for twin-spot *(T)* (Table XII). Six of the F_1 hybrids *(SsT)* were then backcrossed to wild type fish of either species (Tables XIII, XIV). Of 476 backcross hybrids, 224 fish showed single spot and 249 twin-spot, one fish exhibited both patterns, and two possessed none. These results are in good accord with the assumption that the tail spot loci of the two species are on homologous chromosomes that segregate during meiosis.[15]

The exceptional backcross male (1587-11) that exhibited both the single spot and twin-spot patterns, was mated to two wild type females of *X. milleri*. In the second backcross generation to *X. milleri* (Table XV), 19 fish exhibited no tail patterns, 45 fish were single spot, 35 were twin-spot and 8 fish showed both patterns. This result rules out the possibility that *Ss* of *milleri* and *T* of *maculatus* had become linked on the same chromosome as a result of crossing over. The exceptional *SsT* male of the first backcross

[15] Among the hybrids involving *X. milleri* and the Hp-2 strain of *X. maculatus*, a large number of fish with two *X* chromosomes differentiated into functional males (Table XII). Some of these *XX* males became sexually mature *before* their *XY* sibs. In a second series of hybridizations involving the Gp strain of *X. maculatus*, all the *XX* fish developed into females and all but one of the *XY* fish into males (Table X). These crosses well illustrate the difficulty that may be encountered in using data from hybridizations to explain sex-determining mechanisms in *Xiphophorus*.

TABLE XIII. TEST FOR ALLELISM OF TAIL SPOT PATTERNS OF *Xiphophorus maculatus* AND *X. milleri* (BACK-CROSS TO *maculatus*, Gp)

Pedigree	Parents					Offspring (phenotypes)																
	Female				Male					Females					Males							
										Sd *Ss*	*Sd* *T*	*Sp* *Ss*	*Sp* *T*	*Sd* +	*Sd* *Ss*	*Sd* *T*	*Sp* *Ss*	*Sp* *T*	*SpSd* *Ss*	*SpSd* *T*
1603	Gp	X_{Sp}	X_{Sd}	+	1532-11	X_+	Y_{Sd}	*Ss*	*T*	2	8	5	3	..	19	14	10	9	7	13
1605	Gp	X_{Sd}	X_{Sd}	+	1532-13	X_+	X_+	*Ss*	*T*	29	22	..	..	..	21	22	..	..	..	..
1691	1532	X_+	X_+	*SsT*	Gp	X_{Sp}	Y_{Sd}	+		..	..	16	23	2	15	15	..	..	..	..

generation could have resulted from nondisjunction that occurred during oogenesis in its F_1 parent. If this is the case, the offspring of the exceptional *SsT* male should consist of four classes of offspring in the following frequencies: 16.6% wild type, 16.6% showing both patterns, and 33.3% each showing single spot or twinspot. The observed result differs little from the theoretical ratio (Table XV). The deviation can probably be explained by the abnormal segregation that would be expected in a fish that is a species hybrid as well as trisomic. Presumably, the two exceptional wild type offspring of pedigree 1691 (Table XIII) represent a corresponding nullosomic class.

IV. Discussion

The most detailed and extensive analyses of gene and chromosome homologies in macroorganisms have been made with the genus *Drosophila,* and workers with these flies have provided the best discussions of methods, criteria, and pitfalls (Spencer, 1949; Patterson & Stone, 1952, pg. 261, 541; Dobzhansky, 1959). Homologous chromosomes are those similar enough to undergo synapsis during meiosis even in a hybrid, and a basic, but neither essential nor sufficient, criterion for homology between genes is that they lie on homologous chromosomes. That the loci for two similar series of multiple alleles, belonging to different species, can be shown to lie on homologous chromosomes is considered especially strong evidence for the homology of the two loci. Nearly as strong is the case in which single, phenotypically identical, or nearly identical, mutants are found to be located on homologous chromosomes. If two mutants are only somewhat similar, the fact that they are located on homologous chromosomes may nevertheless indicate they are homologous. Mutants that occupy non-homologous chromosomes can only questionably be considered homologous, however, even though their phenotypic manifestations seem to be identical, unless they can be shown to belong to two similarly arranged groups of genes, one of which has presumably become relocated by translocation. In the absence of such detailed linkage maps, association with similar arrangements of only a few genes may serve to make homology more probable, especially when there are numerous chromosomes in the genome. Among the species and subspecies of fishes belonging to the genus *Xiphophorus,* the evidence for gene homology carries all these degrees of weight.

Kosswig (1948, 1961) has discussed gene homology in *Xiphophorus,* especially in relation to evolutionary parallelism and convergence.

TABLE XIV. TEST FOR ALLELISM OF TAIL SPOT PATTERNS OF *Xiphophorus maculatus* AND *X. milleri* (BACKCROSS TO *milleri*)

Pedigree	Parents		Offspring (phenotypes)							
	Female	Male	Females		Males					
			+ *Ss*	+ *T*	*Sd Ss*	*Sd T*	*Sv Ss*	*Sv T*	*Sv Ss T*	+ *SS*	+ *T*
1587	1532 $X_+ X_+$ *Ss T*	1410 - $X_+ Y_{Sv}$ +	13	11	..	..	14	22	1	2	2
1604a	1410 $X_+ X_+$ +	1532-12 $X_+ Y_{Sd}$ *Ss T*	2	2	4	3	..	..	..	1	..
1604b	1628 $X_+ X_+$ +	1532-12 $X_+ Y_{Sd}$ *Ss T*	20	19	10	21	..	..	..	1	..
1606	1544 $X_+ X_+$ +	1532 - $X_+ X_+$ *Ss T*	22	28	..	..	..	..	..	11	12

The sex chromosomes of *Xiphophorus maculatus, X. variatus,* and *X. milleri* are homologous with one another, since they segregate consistently in F_1 hybrids. They possess similar, most probably identical, gene loci; in all three species, the macromelanophore locus is sex-linked, and in *maculatus* and *variatus,* this is linked to a second locus controlling red and yellow pigmentation of body and fins.

Kallman (1965a) recognized that the *X* and *Y* chromosomes of *X. maculatus* and *X. variatus* are homologous with similar genes, and he pointed out that this situation provides very strong evidence for the possession of sex chromosomes by the ancestral form of the two species. That the gonosomes of *X. milleri* also are homologous strengthens this point of view; presumably, the sex-chromosome mechanism of all three species had a common origin in an ancestral form with an *XX-XY* (male heterogametic) system. Sex determination, according to the *XX-XY* scheme, is present today in *X. milleri* and *X. variatus,* but in *X. maculatus* it has evolved further. In this species three types of females *(WY, WX, XX)* and two types of males *(XY, YY)* occur. It has been suggested that populations with the *XX-XY* (male heterogametic) and *WY-YY* (female heterogametic) mechanisms were geographically segregated and that the two systems evolved independently from each other from a

TABLE XV. TEST FOR NONDISJUNCTION IN A BACKCROSS HYBRID OF *Xiphophorus maculatus* x *X milleri*

Parents[1]

1628-1 + + ♀

1587-11 *Sv Ss T* ♂

1602-1 + + ♀

Offspring (ped. 1745)

Female	Male
+ (4)	+ (4)
T (13)	T (2)
Ss (16)	Ss (10)
Ss T (2)	Ss T
Sv +	Sv + (11)
Sv T (1)	Sv T (19)
Sv Ss	Sv Ss (19)
Sv Ss T (1)	Sv Ss T (5)

Phenotypic classes	+	*Ss*	*T*	*Ss T*	Total
Expected	17.8 (16.6%)	35.6 (33.3%)	35.6 (33.3%)	17.8 (16.6%)	107
Observed	19	45	35	8	107
$\frac{(\text{Obs.}-\text{Exp.})^2}{\text{Exp.}}$	0.1	2.7	.01	5.4	$\Sigma = \chi^2 = 8.3$

$n = 3.\ p = 0.05 > 8.3 > p = 0.01.$

[1] See Table XIV for origin of male parent.

polygenic condition (Gordon, 1952; Anders & Anders, 1963). This explanation is now untenable, however, in view of the chromosome homologies discussed above and the discovery that *W* and *X* chromosomes are found together in the same population (Kallman, 1965a). It would have been a remarkable coincidence indeed, if the same pair of autosomes (out of the 24 available pairs) had evolved into sex chromosomes independently in the three species, and that during this evolutionary change the chromosomes had diverged so little that they maintained their specific pairing affinity during meiosis.

None of the macromelanophore alleles of *X. maculatus, X. variatus,* and *X. milleri* is identical, since each produces a different pigment pattern and, as far as known, these differences are maintained and often accentuated when the alleles are introduced into the genomes of other species or subspecies by means of introgressive hybridization. The different patterns cannot be primarily the result of different genetic backgrounds. The *At* and *Sc* genes of *X. montezumae cortezi* are not allelic and there is no evidence that they are sex linked. No critical experiment has yet been published that would test the possibility that the *At* or *Sc* is located on a chromosome homologous with the sex chromosome of *X. maculatus, X. variatus,* or *X. milleri.*[16] At the present time, it is also not possible to determine whether the loci for *At* and *Sc,,* which are evidently located on different chromosomes, are homologous with each other and perhaps also to the macromelanophore genes of the other species of *Xiphophorus.* Since there is evidence that the macromelanophore alleles of *X. maculatus* represent a super-gene (Gordon, 1937a; MacIntyre, 1961c), it is possible that some of the closely linked macromelanophore loci have become separated through translocation during the course of evolution and are now situated on different chromosomes. An alternate possibility, of course, is that the loci of *At* and *Sc* in *X. m. cortezi* are of independent origin. Similar considerations apply to the spotting of *X. hellerii.* This locus may be homologous to the macromelanophore genes of other species, even though it is not now located on a chromosome that is homologous with the sex chromosomes of *X. maculatus.*

All available evidence indicates that the tail spot patterns of *X. maculatus, X. variatus,* and *X. milleri* are also controlled by homologous genes. As is the case with the macromelanophore patterns, it is unlikely that the tail spot genes in the three species would have arisen independently on the same pair of chromosomes —all of which are homologous, pair by pair, since all of them segregate in F_1 hybrids during meiosis. There can be little doubt that the progenitor to which all three species could ultimately be traced already possessed the tail spot locus. There is no evidence yet whether the caudal blotch patterns of *X. montezumae* and *X. p. nigrensis* are homologous with each other or with the tail spot loci of other platyfish.

In some species or subspecies, tail spot patterns occur that are almost identical in appearance and these are perhaps controlled by identical alleles. This can only be established with certainty by comparing the phenotypic effect of the alleles against an identical genetic background. Strikingly similar are the one-spot of *maculatus* and the single spot of *milleri;* the dot of *maculatus* and the point of *milleri;* the simple crescent of *maculatus, v. variatus,* and *v. xiphidium;* the caudal blotch of *montezumae* and *p. nigrensis;* and the upper cut-crescent, cut-crescent, and peduncular spot of *v. variatus* and *v. xiphidium.* In contrast, patterns unique for one form are the moon, moon complete, comet, and complete-crescent of *maculatus* and the bar of *milleri.*

There is as yet no answer to the question why certain patterns are widespread while others occur only in single species or are absent from certain populations of others. Although there are exceptions, the species, subspecies, or populations of *Xiphophorus* with relatively restricted ranges are less polymorphic, as Gordon (1943) pointed out. Neither macromelanophore nor tail spot patterns are known from the two subspecies of *X. couchianus* or from *X. p. pygmaeus.* In *X. p. nigrensis,* only a single tail spot pattern occurs, and in *X. v. evelynae,* only one macromelanophore pattern. These forms all occupy limited areas, especially as compared with *X. maculatus, X. v. variatus, X. v. xiphidium,* and *X. hellerii* (see Text-fig. 1, Table XVI).

According to Gordon & Gordon (1957), the simple crescent and comet patterns are absent from the populations of *X. maculatus* inhabiting the Rio Usumacinta (which lies at the center of this species' distribution) as well as from the rivers in British Honduras (at the southern edge of its range), while moon and moon complete are not known from the Rio Jamapa (at the northern edge). The Rio Jamapa population may have been derived from a chance invasion of platyfish from the Rio Papaloapan, immediately to the south, with the moon and moon complete patterns not represented in the introduc-

[16] There is evidence, however, indicating that *Sc* may be located on a chromosome homologous with the sex chromosome of *X. maculatus* (see page 124).

TABLE XVI. GEOGRAPHY OF THE KNOWN MELANOPHORE POLYMORPHIC PIGMENT PATTERNS IN *Xiphophorus*

Species	No. of Macromelanophore patterns[1]	No. of Tail Spot patterns[1]	Geographic Distribution
X. c. couchianus	None	None	Limited: spring pools of Huasteca Canyon, Nuevo Leon
X. c. gordoni	None	None	Limited: a few small lagunas near Cuatro Cienegas, Coahuila
X. p. pygmaeus	None	None	Limited: Rio Axtla (Rio Panuco system)
X. p. nigrensis	None	1[2]	Limited: Nacimiento del Rio Choy (Rio Panuco system)
X. milleri	2	3[3]	Lake Catemaco
X. clemenciae	None	None	Limited: Rio Sarabia
X. m. montezumae	2[4]	1[2]	Headwater streams of Rio Tamesi and northern tributaries of Rio Panuco
X. m. cortezi	2[4]	1[2]	Headwater streams (southern tributaries) of Rio Panuco
X. v. variatus	4[5]	4[6]	Widespread: Rio Panuco, Rio Tamesi, Estero Cucharas, Rio Tuxpan, Rio Cazones, Rio Tecolutla, Rio Nautla
X. v. xiphidium	2[5]	4[6]	Rio Soto la Marina
X. v. evelynae	1[5]	None	Limited: headwaters of Rio Tecolutla
X. maculatus	7	8[3, 6]	Widespread: Rio Jamapa south to Belize River
X. h. hellerii	None	None	Widespread: Rio Nautla and Rio Jamapa
X. h. strigatus	1	None	Widespread: Rio Papaloapan and Rio Coatzacoalcos
X. h. guentheri	2	1	Widespread: Rio Tonala (Mexico) south to Rio Bonito (Honduras)
X. h. alvarezi	None	None	Limited: Rio Santo Domingo (Rio Usumacinta system)

[1] Not including the absence of any pattern, that is, the so-called wild type.

[2] The tail spot pattern of *X. p. nigrensis*, *X. m. montezumae*, and *X. m. cortezi* appears to be identical.

[3] Two patterns of *X. milleri* are identical, or nearly identical, with two of *X. maculatus*.

[4] The macromelanophore patterns of *X. m. cortezi* are not identical with those of *X. m. montezumae*.

[5] The macromelanophore patterns of each of the three subspecies of *X. variatus* are distinctive.

[6] One pattern, simple crescent, appears to be identical in *X. v. variatus*, *X. v. xiphidium*, and *X. maculatus*. Three other patterns are shared by *X. v. variatus* and *X. v. xiphidium*.

tion. This explanation could also account for the absence of the *N* gene from the Jamapa. On the other hand, it may be significant that of the eight tail spot patterns of *X. maculatus*, two that are most similar to each other, moon and moon complete, are both absent from the Rio Jamapa. Perhaps selection has been a factor in the elimination of these two similar patterns from the Jamapa population. The absence of comet and simple crescent from the Rio Usumacinta is more difficult to understand. Neither chance migration nor genetic drift appears to be a likely explanation.

The pattern complete-crescent *(Cc)* of *X. maculatus* might be considered to be composed of two single patterns, simple crescent *(C)* and axhead, both of which also occur by themselves. The former is common in certain populations (Gordon & Gordon, 1957), but the latter is extremely rare (Gordon, 1947b). Another pattern of *X. maculatus* that might be a composite is moon complete *(Mc)*. Although crossing over within the tail spot locus has never been observed, the tail spot patterns may well comprise a super-gene, as do many of the series of dominant multiple alleles that produce polymorphism (Ford, 1964, 1965).[17] This view is supported by the discovery of two *X. maculatus* in which complete-crescent and dot were inherited as a unit and one in which complete-crescent and

[17] See footnote 3, page 110.

one-spot were so inherited. Composite tail spot patterns are not known from any other species of *Xiphophorus*.

The caudal pigment spot, the grave, of the Rio Chajmaic swordtail does not resemble any of the tail spots of other species. The fin rays involved in this pattern are the same ones that form the dorsal edge of the sword and become pigmented, although to a much lesser degree, in the males of previously described populations of *X. hellerii*. When such males are examined, one finds that the two or three fin rays immediately above the dorsal edge of the sword are pigmented, and that the pigmentation of each one ends progressively more anteriorly. This is an arrangement almost identical to that of the grave in female and immature swordtails of the Rio Chajmaic.[18] Although females of other swordtail populations do not exhibit the grave pattern, they will develop a sword, edged typically with black, when exposed to androgens (Dzwillo, 1962). The significant fact about the swordtail population from the Chajmaic may not be that both male and female fish have seemingly become homozygous for a tail pattern allele, but that they have evolved a genetic system that has freed this pattern from the control of androgenic hormone. This change would not have been a simple one and must have involved an intensification of the pigmentation. Although the same fin rays are involved, the dorsal edge of the sword, especially the proximal portion, is much darker and appears wider in the Rio Chajmaic population than in the other swordtail populations.

Although the study of gene homologies among members of the genus *Xiphophorus* provides pertinent information on the evolution of these fishes, it cannot serve as sole arbiter in deciding their phylogenetic relationships. For example, Rosen (1960) found *X. maculatus* and *X. variatus* so closely related that he considered them to constitute a well defined superspecies. The homologous macromelanophore and tail spot genes of these two species might therefore be considered to corroborate their close evolutionary relationship, but a third species, *X. milleri*, that also possesses the same two homologous sets of alleles was placed in a different, less closely related, species group by Rosen (1960) on the basis of an array of morphological and ecological characters.

Little work has been done on gene or chromosome homologies in other genera of poeciliids. In *Poecilia, P. sphenops* and *P. latipinna* possess similar mottled and solid black pigmentation and, in this respect, differ from all other members of the genus. Schröder (1964) showed that the solid black phenotype of *P. sphenops* results from the additive effect of two loci (*M* and *N*) that can combine freely. In *P. latipinna*, however, a single locus is concerned with black pigmentation. According to Schröder, the pigment gene of *latipinna* is homologous to *M* of *sphenops*, but this has not been definitely established (see table 16, pg. 410 of Schröder, 1964). The subgenus *Lebistes* is characterized by a high degree of color polymorphism in adult males (Rosen & Bailey, 1963). In addition to the well known guppy, *Poecilia reticulata*, five species are currently recognized, and the males of at least the majority of them exhibit spots of many shapes and colors on the body and dorsal and caudal fins. In *P. reticulata*, most of these patterns are controlled by sex-linked genes (Haskins *et al.*, 1961), but virtually nothing is known about their inheritance in the other species. Pigmentary polymorphism has been described in other poeciliids, sometimes in the form of black-spotted individuals, *e.g.* in *Gambusia, Phalloceros*, and *Girardinus* (Myers, 1925). Again, nothing is known about the inheritance of these patterns, although black-spotting and melanism are usually confined to males in *Gambusia affinis* (Myers, 1925; Regan, 1961). Because of the light that may be shed on the evolution of sex-determining mechanisms in the Family Poeciliidae, possible sex linkage and chromosome homology ought to be investigated wherever possible among these forms. Whatever the outcome of such investigations, they will have important bearing on the understanding of sex determination in fishes.

V. Summary

1. Fishes of the poeciliid genus *Xiphophorus* can be hybridized with each other in the laboratory, and the hybrids are fertile to a significant degree. Most of the species are polymorphic for pigment patterns that are controlled by major genes. These characteristics make *Xiphophorus* especially suitable for the detection and study of gene and chromosomal homologies.

2. Eighteen polymorphic pigment patterns formed by macromelanophores and 16 formed by micromelanophores are reviewed. Patterns previously unrecognized are described in detail and their mode of inheritance is analyzed. A uniform system of nomenclature is applied to the patterns, all previously used terms being recorded and, if necessary, synonymized.

[18] Some male *X. pygmaeus nigrensis*, in which subspecies the dorsal margin of the caudal sword is not edged with black, exhibit a pattern similar to grave.

3. The sex chromosomes of *X. maculatus, X. variatus,* and *X. milleri* are homologous, and loci on them are occupied by homologous genes that control the macromelanophore patterns.

4. It is highly probable that the multiple, dominant alleles that control the series of similar micromelanophore tail patterns of *X. maculatus, X. variatus,* and *X. milleri* are homologus.

5. The homologies of the other pigment pattern genes are not known, but some of them have been shown to occur on non-homologous chromosomes.

6. That the sex chromosomes of three species of *Xiphophorus* are homologous strongly indicates the existence of a common ancestor with the same type of sex-determining mechanism *(XX-XY).*

7. The macromelanophore patterns of all species and subspecies are phenotypically distinct. Hybridization either demonstrates or strongly indicates that these differences are not the result of modifying genes, but depend on the principal genes themselves.

8. Three possible cases of crossing over within the locus for tail spot patterns in *X. maculatus* were discovered. The probability that these multiple alleles comprise a super-gene, or some similar arrangement, is thus increased.

9. Both sexes of one population of *X. hellerii* exhibit a micromelanophore tail pattern that closely resembles a secondary sex character associated with the "sword" of the adult males in other populations; possibly a change in the genetic system has freed this pattern from the control of male sex hormone.

10. In general, the species or subspecies of *Xiphophorus* with extensive geographic ranges are more polymorphic than those with restricted ones.

11. A case of non-disjunction in a hybrid fish *(X. milleri* x *X. maculatus)* is described.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. *Xiphophorus variatus variatus.* Female (ped. 1671), an eleven and a half month old fish in which the spots of the macromelanophore pattern, punctatus *(P²)*, have coalesced to form an irregular black band, The micromelanophore pattern, crescent *(C)* is also shown.

Fig. 2. Backcross hybrids, *(maculatus* x *couchianus)* x *couchianus.* Female, above (ped. 1166) has one-spot *(O).* Male, below (ped. 881), has dot *(D).* These tail spot patterns have maintained their distinctiveness. See Table VIII.

Fig. 3. *Xiphophorus pygmaeus nigrensis.* Male, above (ped. 1813), and female, below (ped. 1815), have the tail spot pattern, caudal blotch *(Cb).* Male, center (ped. 1813), is wild type, that is, has no tail spot pattern.

Plate II

Fig. 4. *Xiphophorus milleri.* Male, lower left (ped. 1748), has macromelanophore pattern, spotted ventral *(Sv,)* and micromelanophore tail spot patterns, point *(Pt)* and bar *(B).* Female, upper left (ped. 1748), has point. Female, upper right (ped. 1628), has tail spot pattern, single spot *(Ss).* Female, lower right (ped. 1602), has bar.

Fig. 5. *Xiphophorus milleri.* Male, above (ped. 1602), has bar *(B).* Female, left, below (ped. 1628), has single spot *(Ss),* which appears bar-like because of a high light. Female, right, below (ped. 1601), has point *(Pt).* The gonopodium of the male is pigmented with micromelanophores.

Plate III

Fig. 6. *Xiphophorus maculatus.* Male, left (Cp-11), shows two tail spot patterns, complete-crescent *(Cc)* and dot *(D).* Female, right (Jp-30[10]), shows the one-spot *(O)* for which it is homozygous.

Fig. 7. *Xiphophorus maculatus.* This female (ped. 270) is one of the offspring of the fish in Fig. 6. It shows the complete-crescent *(Cc)* and one-spot *(O)* patterns. See Table VIII. For a discussion of the effect of intraspecific hybridization on the macromelanophore pattern, spotted dorsal *(Sd),* see Gordon (1951a).

Fig. 8. *Xiphophorus maculatus,* Hp-1 strain. Both fish have the tail spot pattern, complete-crescent *(Cc),* and lack the dot *(D).* The female, above, shows a typical manifestation of the macromelanophore pattern, spotted dorsal. The male, below, has a prominent slash mark, a micromelanophore pattern whose mode of inheritance is not known.

Plate IV

Fig. 9. *Xiphophorus hellerii guentheri,* Bx strain. Both male and female have the macromelanophore pattern, dabbed *(Db[1]).*

Fig. 10. *Xiphophorus hellerii guentheri,* Hx strain. The male has the macromelanophore pattern, dabbed *(Db[2]),* in which the spots are typically arranged in rows.

Plate V

Fig. 11. *Xiphophorus hellerii,* Ch strain. The tail spot pattern (grave) of the female (below) and the heavy pigmentation of the dorsal edge of the caudal sword of the male are characteristic of this form.

Fig. 12. *Xiphophorus variatus xiphidium.* Male, left (ped. 1711), has macromelanophore pattern, flecked *(F1[1]),* while female, right (ped. 1708 or 1758), has dusky *(F1[2]).* Both fish have the tail spot pattern, peduncular spot *(Ps),* and in the male, its extension behind the hyplural bone is clearly evident.

Fig. 13. *Xiphophorus montezumae montezumae* (ped. 1817) with micromelanophore pattern, caudal blotch *(Cb).* Cross section through the proximal portion of the caudal fin showing dense accumulations of pigment cells in the dermis between the musculature and scales, between the scales, and along the connective tissue that lies between the dorsal and ventral edges of the lepidotrichia and the dermis. X 65.

Plate VI

Fig. 14. *Xiphophorus variatus xiphidium* (ped. 1792) with micromelanophore pattern, peduncular spot *(Ps).* Cross section through the last caudal vertebra. In contrast to the one-spot pattern *(O)* of *X. maculatus,* the pigment cells are located in the deep-lying muscles, especially around blood vessels and nerves. X 65.

Fig. 15. *Xiphophorus variatus xiphidium* (ped. 1792) with micromelanophore pattern, peduncular spot *(Ps).* Cross section at the level of the hyplural plate. Pigment cells are located between the deep-lying muscles and around the blood vessels. X 130.

Fig. 16. *Xiphophorus maculatus* (Np strain) with micromelanophore pattern, moon *(M).* Cross section through the last vertebra. Pigment cells are located in the dermis and the superficial muscles immediately underneath. X 65.

Fig. 17. *Xiphophorus maculatus* (Np strain) with micromelanophore pattern, moon *(M).* Cross section at the level of the hyplural plate. Pigment cells are located in the dermis and between the superficial muscles. X 130.